Optimization of foam mat drying process of moringa leaf powder (Moringa oleifera) as protein and amino acids sources

Wahyuni, R., Wignyanto, W., Wijana, S. and Sucipto, S

1Department Food Science and Technology, Faculty of Agriculture, Yudharta University, Purwosari, Pasuruan, East Java, Indonesia
2Department of Agro Industrial Technology, Faculty of Agricultural Technology, Brawijaya University, Malang, East Java, Indonesia

Abstract
Moringa (Moringa oleifera) is a well famous plant that grows in almost all parts of Indonesia. These plants have many benefits in the fields of food, cosmetics, and health. The components of moringa that can be utilized are the nutrients and the bioactive compounds. One of the nutrients that are required by the human body is protein. This protein can be taken through the extraction method, but unfortunately, the extract has several drawbacks because it is easily rotten. As a result, further methods are needed to maintain the shelf life and product quality, namely microencapsulation using foam mat drying method. The present study was intended to obtain the optimum concentration of maltodextrin, tween 80, and drying temperature in microencapsulation using the foam mat drying method so that the protein and amino acids in Moringa leaf protein concentrate powder can be protected. Besides, it also was aimed at determining the digestibility of the Moringa leaf protein concentrate powder. This study consisted of three factors, namely X1 (Tween 80 concentration) which contained three levels, namely 0.1, 0.2, 0.3%; X2 (maltodextrin concentration) which had three levels, namely 5, 10, 15%; and X3 (foam mat drying temperature) consisting three levels, namely 50, 55, 60°C which were arranged using the Response Surface Method (RSM) with the Central Composite Design (CCD). The research parameters utilized were total yield, total protein, amino acid content, and protein digestibility. The results of the drying process optimization were tween 80 concentrations of 0.201%, maltodextrin up to 13.79%, and the temperature was 53.46°C with a total yield of 25.17% and a protein content of 25.377% with the desirability of 0.846. The combination of these treatments also produces 20 amino acid components in Moringa leaf powder and the protein digestibility is quite high, namely 62, 856%.

1. Introduction
Moringa (Moringa oleifera) is a plant of the Moringaceae family that is easily grown in tropical and sub-tropical areas. This plant grows in almost all parts of Indonesia. Many people utilize moringa plants as a hedge plant and vegetable material (Siddharaju and Becker, 2006). Many research data have reported the function of moringa in the health and beauty sector. According to Chumark et al. (2008), moringa functions as a hypolipidemic, antiatherosclerotic, and prevents cardiovascular disease. Meanwhile, according to Mahmood et al. (2010), moringa can be used as an anti-diabetes, inflammation, anemia, and improving the immune system. This is because moringa is rich in nutrients, essential amino acids, vitamins, minerals, and a source of natural antioxidants. Dried leaves contain 27.2% protein, 17.1% fat, 5.9% water content, and 38.6% carbohydrates (Yameogo et al., 2011) which are equivalent to 9 yogurt proteins, 10 vitamins A carrots, 25 iron spinach, 15 potassium bananas, 17 milk calcium, and 7 vitamin C oranges (Rockwood et al., 2013).

In addition, to be rich in nutrients, Moringa oleifera also contains bioactive compounds, including complex flavonoids consisting of glucosides, routineosides, malonylglicosides, acetylglucoside kaempferol, quercetin, isorhamnetin. The leaves are rich in palmitic acid, linolenic acid, oleic acid seeds. The roots are palmitic acid and oleic acid. The stem and the twigs contain palmitic acid. The entire plant tissue contains potassium, magnesium, and calcium (Amaglo et al.,...
Taking nutrients and bioactive compounds from moringa plants can be done by the extraction method. Extraction is one of the simplest methods whose supporting materials are easily found. But the extracted product is generally in the form of a liquid. The weaknesses of the liquid product are that it is easily damaged, the shelf life is short, the application is limited, and the packaging price is expensive so that further treatment must be conducted to maintain product quality during storage, namely drying and pulverizing. The advantages of pulverization are more stable nutrition, long shelf life, easy distribution, practicality, and wide application of use (Latifah and Apriliawan, 2009; Syahputra, 2014). Moringa leaf protein concentrate extract in this study needed to be dried and powdered so that amino acids and bioactive compounds could be preserved during storage.

Pulverization is influenced by method, the concentration of filler, and drying time (Syahputra 2008). It is necessary to select a suitable drying method so that the product does not lose bioactive compounds. The advantages of foam mat drying are that it uses a low temperature and a short time so that the product does not damage. Besides, taste, colour, and nutrition can be maintained, simple equipment, and low investment costs. Tween 80 is chosen as a foaming agent because it functions as a good food additive, capsule, and emulsifier (Ramadhia et al., 2012; Sulaksono et al., 2013). The objective of this study was to obtain the optimum concentration of maltodextrin, tween 80, and drying temperature in microencapsulation using the foam mat drying method, so that the protein and amino acids in moringa leaf protein concentrate powder can be protected. Besides, it is also intended to determine the digestibility of the moringa leaf protein concentrate powder.

2. Materials and method

2.1 Materials

The materials needed in this study were fresh Moringa oleifera leaf extract, obtained from Sekarmojo Village, Purwosari District, Pasuruan Regency, East Java, extracted using water solvent, Tween 80, Maltodextrin obtained from Medilab Malang, Aquades, CuSO4, Na2SO4, concentrated H2SO4, 50% NaOH, HCl, litmus paper, Na-acetate, Na-EDTA, Methanol, and THF. While the tools are oven, digital scale XP-1500, mixer, volumetric flask, and HPLC (High-Performance Liquid Chromatography).

2.2 Research procedure

This research procedure consisted of 1) Mixing the optimum extract from moringa leaves, Tween 80, and maltodextrin according to the treatment, 2) Shaking the mixture using a mixer for 10 mins with a maximum speed of 1,400 rpm to get foam, 3) The foam mat drying process of Moringa leaf extract uses the temperature according to the treatment, 4) Analyzing yield, total protein, amino acid content, and protein digestibility.

2.3 Foam mat drying process

The optimum extract of moringa leaves which was extracted using water solvent was mixed with tween 80 (concentration 0.1, 0.2, 0.3%), maltodextrin (concentration 5, 10, 15%), and dried at temperatures of 50, 55, and 60ºC.

2.4 Experimental design for optimization of Moringa leaf powder using Response Surface Methodology (RSM)

The experiment employed the Response Surface Method (RSM), which consisted of 3 factors with a mathematical equation model as follows:

\[ r = \beta_0 + \sum \beta_iX_i + \sum \beta_{ij}X_iX_j + \varepsilon \quad (1) \]

Y is the response (result), \( \beta_0 \) is the constant, \( \beta_i, \beta_{ij} \) is the coefficient of the independent variable (X), X is the independent variable with no code. \( X_1 \) is the concentration variable of Tween 80 (0.1; 0.2; and 0.3%), \( X_2 \) is the concentration of maltodextrin (5, 10, and 15%); \( X_3 \) is drying temperature (50, 55, and 60ºC) and \( \varepsilon \) is random errors.

2.5 Yield content analysis

The yield content of Moringa leaf powder can be calculated using the following formula:

\[ \% \text{ Yield} = \frac{\text{weight of powder (final)}}{\text{weight of simplicia (initial)}} \times 100 \% \quad (2) \]

2.6 Protein content analysis

The total protein content of Moringa leaf powder resulting from foam mat drying is analyzed using the Kjeldahl method (Sudarmadji et al. 2007). A powder sample of 1-2 g of material was added with 10 g of silen (a mixture of CuSO4 and Na2SO4). The mixture was put into a Kjeldahl flask and 30 mL of concentrated H2SO4 was added. Then the sample was heated using an electric heater until the solution becomes clear, then it was cooled to room temperature. Then the solution was added with 250 mL of distilled water and 50 mL of 50% NaOH until it had basic character, which is indicated by the change in the colour of the litmus paper from red to blue. The Kjeldahl flask in the distillator was heated until
the ammonia evaporates. The results of the distillation were stored in an Erlenmeyer that had been filled with 25 mL of 0.2 N HCl solutions until 100 mL of distillate was obtained. Then it was titrated using the phenolphthalein indicator until a pink colour was obtained. Apart from that, a blank solution was also prepared which was obtained in the same way. The total protein content was calculated using the following formula:

\[
\% \text{ Total N} = \frac{\text{ml NaOH Blank} - \text{ml NaOH sample}}{\text{n NaOH x 14088 x 109 n Materials In grams x 1000}}
\]  

The protein content was obtained by multiplying the % total N by the conversion factor. The conversion factor was 6.25.

\[
\text{Protein Content (\%)} = \% \text{ total N x Conversion Factor}
\]  

**2.7 Analysis of amino acid content of Moringa Leaf Powder**

Analysis of amino acid content of *Moringa leaf* powder was carried out using the reference method from AOAC (1995) and the ICI Instrument manual (1985). An amino acid analysis was performed using HPLC, by freeing amino acids from protein through hydrolysis with HCl 6 N. The hydrolyser was dissolved with sodium citrate buffer and each amino acid was separated using HPLC.

**2.8 In vitro digestibility test**

The digestibility test was started by taking 1 to 2 g of moringa leaf powder as a sample, 15 mL of 0.1 N HCl is added and put into a 50 mL Erlenmeyer. The HCl solution contained 15 mL of the enzyme pepsin. Then the Erlenmeyer was stirred in a water bath shake at 30°C at low speed for 3 hrs. After that, the solution is neutralized to pH 7 using 0.5 N NaOH and added 4 mL of pancreatic enzymes in 7.5 mL of 0.2 M phosphate buffer solution with pH 8 containing 0.005 M sodium azide. The obtained solution was stirred again in a water bath shake at 37°C at a slow speed for 24 hrs. The solution was filtered with Whatman paper and weighed together. The solid and filter paper was dried in an oven at 105°C for 2 hrs. The oven-dried solids were analyzed for nitrogen content using the Kjeldahl method. The obtained protein is called residual protein (Muchtadi, 1993). The in vitro digestibility calculation is obtained from the following formula:

\[
\text{Protein Digestibility (\%)} = \frac{\text{N total in sample} - \text{N in residue}}{\text{N total in sample}} \times 100 \%
\]  

**2.9 Data analysis**

Data on amino acid content and digestibility were analyzed descriptively. While the yield data and protein content were analyzed using the MINITAB Release 14 software. Based on this analysis, a graph of the responses of each factor and the interaction between factors would be obtained in the form of a 3D response surface plot and contour plot (using Design expert 7), to test the model from optimum foam mat drying.

**3. Results and discussion**

Research data consisting of total yield and protein content data are presented in Table 1. Table 1 shows that the yield value of moringa leaf powder resulting from foam drying which using variations in the concentration of Tween 80, maltodextrin, and the lowest drying temperature with the concentration of Tween 80 was 0.2%; maltodextrin 1.59%; and the drying temperature of 55°C with a yield value of 5.410%. While the highest yield was 27.490% at the concentration of Tween 80 was 0.2%; maltodextrin 18.41%; and the drying temperature of 55°C. The highest protein content in the Tween 80 concentration was 0.3%; maltodextrin 15%; and the drying temperature was 50°C, and the lowest was 0.03% at the Tween 80 concentration; maltodextrin 1.59%; and the drying temperature of 55°C. The response of each variable is described below.

**3.1 Yield content of Moringa leaf powder (%)**

Yield is one of the important parameters in the research, which states the ratio of the dry weight of the product to the weight of the raw material. The yield of moringa leaf powder is calculated based on the ratio of the final weight (weight of produced powder) to the initial weight (weight of the materials) multiplied by 100% (Yuniarifin et al., 2006). The higher the success rate of the foam mat drying process, the better the yield and quality of the produced powder.

The lowest yield value of moringa leaf powder from foam drying was 5.410%, which was obtained at the concentration of Tween 80 treatment of 0.2%; maltodextrin 1.59%; and a drying temperature of 55°C. While the highest yield was 27.490% at the concentration of Tween 80 treatment of 0.2%; maltodextrin 18.41%; and a drying temperature of 55°C. Based on the results of the analysis, the value of R-square / R² (coefficient of determination) in ANOVA had a value of 0.976. This shows that the data can support a model of 97.6% which includes the treatment of Tween 80, maltodextrin, and drying temperature. The remaining 2.34% was influenced by other factors that were not included in the model. Other factors that affect the total yield of foam mat drying powder were the type of drying method, plant varieties, plant growth environment, and the harvesting age of the moringa plants. The R (Adj R-Squared) value in ANOVA was 0.955, indicating that there is a correlation of 95.5%. According to this response surface method, the polynomial equation of the second-order model was
obtained with the following coded equations:

The quadratic regression model is as follows:

\[ \text{Yield} = -58.94 - 30.09 X_1 + 3.39 X_2 + 2.11 X_3 - 0.115 X_1 X_2 + 0.845 X_1 X_3 - 0.0084 X_2 X_3
- 35.845 X_1^2 - 0.0703 X_2^2 - 0.02 X_3^2 \]  
(6)

Where \( Y_1 \): Yield response variable, \( X_1 \): Tween 80 factor, \( X_2 \): Maltodextrin factor, and \( X_3 \): Drying temperature factor

The above equation shows that the yield response will increase if the Tween 80 concentration decreases, and the maltodextrin concentration and drying temperature increase. Based on the results of the variance analysis, the \( R \) squared value was 0.976, which means that 97.6% of the total variety is included in the yield value so that the model can explain the actual conditions of the concentration of tween 80 and maltodextrin, as well as the drying temperature of the yield of \textit{Moringa} leaf powder.

ANOVA results on the treatment of Tween 80, maltodextrin, and drying temperature did not have a significant effect on the model. However, quantitatively, the addition of the concentration of Tween 80, maltodextrin, and drying temperature will cause an increase in the yield. A graph of the yield levels due to the Tween 80 treatment is presented in Figure 1a, the effect of maltodextrin is presented in Figure 1b, and the effect of drying temperature is presented in Figure 1c.

Figure 1b and 1c have the same trend, where the yield content will slowly increase with increasing concentration of Tween 80 and drying temperature.

| Run | Tween 80 | Maltodextrin | Drying Temperature | Yield (%) | Protein (%) |
|-----|----------|--------------|--------------------|-----------|-------------|
| 1   | 0.10     | 5.00         | 60.00              | 8.23      | 19.411      |
| 2   | 0.20     | 18.41        | 55.00              | 27.49     | 26.528      |
| 3   | 0.20     | 10.00        | 63.41              | 19.51     | 18.196      |
| 4   | 0.30     | 5.00         | 50.00              | 8.12      | 18.340      |
| 5   | 0.03     | 10.00        | 55.00              | 20.37     | 22.850      |
| 6   | 0.20     | 1.59         | 55.00              | 5.41      | 12.987      |
| 7   | 0.20     | 10.00        | 55.00              | 20.41     | 22.798      |
| 8   | 0.20     | 10.00        | 55.00              | 20.49     | 21.050      |
| 9   | 0.37     | 10.00        | 55.00              | 20.45     | 24.652      |
| 10  | 0.20     | 10.00        | 55.00              | 20.38     | 23.613      |
| 11  | 0.10     | 15.00        | 60.00              | 25.11     | 22.693      |
| 12  | 0.20     | 10.00        | 55.00              | 20.35     | 23.291      |
| 13  | 0.20     | 10.00        | 55.00              | 20.43     | 22.532      |
| 14  | 0.30     | 15.00        | 60.00              | 25.46     | 25.705      |
| 15  | 0.20     | 10.00        | 46.59              | 20.50     | 22.693      |
| 16  | 0.10     | 5.00         | 50.00              | 9.25      | 17.362      |
| 17  | 0.10     | 15.00        | 50.00              | 25.27     | 25.219      |
| 18  | 0.30     | 15.00        | 50.00              | 25.61     | 27.311      |
| 19  | 0.20     | 10.00        | 55.00              | 20.50     | 22.870      |
| 20  | 0.30     | 5.00         | 60.00              | 10.51     | 15.975      |
Although the increase was very small, it can be seen that the optimal yield was achieved when the Tween 80 concentration was 0.2% and the temperature was 55°C. Then along with the increase of Tween 80 concentration, the yield would decrease when the concentration continued to increase. This was because the higher the concentration of Tween 80, the higher the total solids and the heavier the obtained product so that the resulting yield is higher (Cheong et al., 2014).

Wijana et al. (2013) state that the higher the drying temperature, the lower the amount of yield. The decrease in yield was caused by the increase in temperature. As a result, more water content evaporated and decreased yields.

Figure 1a explains that the addition of maltodextrin concentration causes an increase in the yield. This is supported by Adhikari et al. (2009) in Largo et al. (2015) who state that maltodextrin can form a gel and the strength to hold water in the product so that the addition of maltodextrin will cause an increase in the amount of yield. Hendrawati et al. (2017) report that the higher the concentration, the lower the yield of the product. This is due to the nature of maltodextrin, which can bind water to the product which increases the total amount of solids so that the yield increases. Besides, maltodextrin is a solid material, so that the addition of maltodextrin to the material can increase the total solids in food that will be dried (Wuryantoro and Wahono, 2014). Likewise, the opinion to Igual et al. (2014), the yield will increase linearly with increasing filler concentration and increasing temperature during drying, but it will decrease again after increasing the temperature.

3.2 Protein content

Protein determination of Moringa leaf powder resulting from foam mat drying was carried out using the Kjeldahl method. Based on Table 1, the highest protein content is 27.311% with a tween 80 concentration of 0.3%; maltodextrin 15%; and the drying temperature was 50°C. The value of R-square/R^2 (coefficient of determination) in ANOVA analysis results was 0.961, or the data can support a model of 96.1%, and the value of R (Adj R-Squared) in ANOVA was 0.926 indicating that there is a correlation of 92.6%. According to this response surface method, the polynomial equation of the second-order model was obtained with the following coded equations:

The quadratic regression model is as follows:

\[
\begin{align*}
\text{Protein Content} = & -84,027 + 16,06 X_1 + 2,23 X_2 + \\
& 3,44 X_1 + 1,89 X_1 X_2 - 0,873 X_2 X_3 - 0,019 X_2 + \\
& 43,079 X_1^2 - 0,039 X_2^2 - 0,029 X_3^2
\end{align*}
\]  

(7)

Where Y_1: Yield response variable, X_1: Tween 80 factor, X_2: Maltodextrin factor, and X_3: Drying temperature factor

Equation 7 describes that the protein content will increase if the concentration of Tween 80, maltodextrin, and the drying temperature increase. The response of each variable is as follows.

Figures 2a and 2b explain that the addition of the concentration of Tween 80 and maltodextrin will cause an increase in protein level. Although based on ANOVA treatment, the concentration of tween 80 and maltodextrin was deemed not significant, but quantitatively, there was an increase in the amount of protein during Tween 80 and the concentration of maltodextrin was increased. An increase in the concentration of Tween 80 which was used as a foaming agent in the manufacture of moringa leaf powder, Figure 2a shows that the increase in the concentration of tween 80 from 1% to 3% caused the amount of protein to continue to increase. This was because the higher the
concentration of Tween 80, the higher the formation of foam. The foam makes water absorbs easily when shaking and mixing before drying, so that during the drying process, the water content of the material will disappear faster and smaller. As a result, the amount of protein in the material increases (Kumalaningsih, 2005). Sebranek (2009) further explains that the high or low protein value can be influenced by the amount of water lost (dehydration) of the material. The protein value will be greater if the amount of water lost is greater.

Figure 2b shows that the addition of maltodextrin concentration will cause an increase in the amount of protein. This is because the ability of maltodextrin has a strong binding power to the coated material and can protect the proteins contained in the material (Oktaviana, 2012). According to Mishra and Mahanta (2014), the use of maltodextrin in the encapsulation process can bind nutrients, including the proteins contained in the ingredients. Whereas in the drying temperature treatment, it appeared that the more the temperature was raised, the protein content decreased. This is because increasing the temperature of the foam mat drying will cause protein denaturation. This is in line with the opinion of Yuniarti et al. (2013) in Lisa et al. (2015), which states that heating can damage amino acids and cause denatured proteins.

3.3 Optimization of yield response and protein content

Optimization results employ Design Expert Ver. 9. The trial which shows that the optimal conditions for the foam mat drying process of Moringa leaf powder are the treatment of tween 80 up to 0.201%, maltodextrin 13.79%, and a temperature of 53.46°C. This treatment will produce a response prediction in the form of a total yield of 25.17%, and protein content of 25.377% with the desirability of 0.846 or 84.6%. The contour and 3D plot graphs are presented below.

Figure 3 shows that the increase in the concentration of tween 80 and maltodextrin causes an increase in the yield and protein content. This is in contrast to the drying temperature treatment. The red colour showed the highest value of the yield and protein response. In the drying process using the foam mat drying method, the process did not require high temperatures, because the tween 80 would encourage the formation of foam. After all, it will enlarge the surface so that it speeds up drying. While the higher the temperature will damage the protein material, so that the higher the temperature, the lower the protein content.

While the desirability value showed the desired scale for each response and determined the degree of accuracy of the optimal solution results. The scale range for desirability values was from 0-1, where a value of 0 indicates a fully unwanted response, while a value of 1 indicates a fully desirable response (Bezerra et al., 2008). In this study, it had a desirability of 0.846 or 84.6%, where the value was closer to the value of 1, which means the higher the value of the optimization accuracy.

3.4 Amino acid content of Moringa leaf powder

The quality of the protein in a material is determined by the level of amino acids contained in the material, so amino acid analysis is very important to conduct. Amino acids, when viewed from a nutritional perspective, consist of two groups, namely essential and non-essential amino acids. Essential amino acids are amino acids that cannot be produced by the body itself and must be obtained from food sources of protein. Meanwhile, non-essential amino acids are amino acids that can be produced by the human body. The amount of essential amino acids that must be available in the food that we consume is at least fifteen kinds of amino acids, namely phenylalanine, tyrosine, isoleucine, lysine, methionine, cystine, threonine, valine, tryptophan, arginine, histidine, glycine, serine, asparagine, and proline (Mandila and Hidajati, 2013).

There were twenty amino acids in Moringa leaf powder resulting from foam mat drying as seen in Figure 4 and Table 2.
Based on Figure 4 and Table 2, it can be seen that Moringa leaf powder contained twenty amino acids, consisting of nine essential amino acids and eleven non-essential amino acids. The nine essential amino acids were threonine (7041.83832 µg/g), Valine (15301.47228 µg/g), Methionine (4795.69096 µg/g), Isoleucine (825.526598 µg/g), Leucine (11704.34076 µg/g), Phenylalanine (5719.83016 µg/g), Histidine (11329.1744 µg/g), Lysine (15726.06126 µg/g), and Tryptophan (2219.54373 µg/g). The highest essential amino acid content was in Valine at 15301.47228 µg/g and the lowest was in Tryptophan at 2219.54373 µg/g. Meanwhile, non-essential amino acids consisted of aspartate acid (16740.53531 µg/g), glutamic acid (22030.21174 µg/g), Serine (22030.21174 µg/g), and Asparagine (1334.53177 µg/g).

### 3.5 Protein digestibility of Moringa leaf powder

Protein digestibility is the ability of proteins to be hydrolyzed into amino acids by digestive enzymes (Guo et al., 2007; Cisse et al., 2013). Enzymes only react with one substrate (protein) and change the substrate into one product, so that the higher the protein content, the greater the protein digestibility. The following is the digestibility of the moringa leaf powder based on the result analysis.

Table 3 shows the protein digestibility of moringa leaf powder was 62.856%. Protein digestibility is influenced by several factors, including anti-nutritional factors such as anti-trypsin and anti-chymotrypsin. Besides, the occurrence of reactions between proteins...
(amino acids) and other components can also affect protein digestibility. Poorly controlled processing or preservation of protein food can also reduce the nutritional value of protein, for example, the process of crushing, heating, cooking, and drying. All of this can cause a decrease in the nutritional value of protein due to decreased protein digestibility and decreased availability of essential amino acids. A protein that is easily digested indicates that the amount of amino acids that can be absorbed and used by the body is high. On the other hand, a protein that is difficult to digest because most of it will be excreted by the body with faeces (Saputra 2014).

Table 3. The digestibility (%) of Moringa Leaf Powder

| Sample                         | Number | Digestibility (%) |
|--------------------------------|--------|-------------------|
| Moringa Leaf Powder            | 1      | 63.074            |
|                                | 2      | 62.639            |
| **Average**                    |        | 62.856            |

4. Conclusion

Based on the research results, it can be concluded that the optimum condition of the foam mat drying process that can protect protein and amino acids of Moringa leaf powder is at a concentration of tween 80 of 0.201%, maltodextrin up to 13.79%, and a temperature of 53.46°C. At this concentration, the total yield is 25.17%, and protein content is 25.377% with the desirability of 0.846 or 84.6%. The combination of these treatments also produces twenty amino acid components contained in Moringa leaf powder and the protein digestibility is quite high, namely 62.856%.

Conflict of interest

The authors declare no conflict of interest.

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