NUMERICAL OPTIMIZATION OF POLY(LACTIC ACID) COATED CASSAVA LEAF BIOCOMPOSITE SHEETS USING RESPONSE SURFACE METHODOLOGY

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Response surface methodology (RSM) was used to optimize the tear index of newly developed biocomposite packaging sheets made up of cassava leaves and poly(lactic acid) (CL/PLA). The effect of process parameters, such as PLA concentration (6-10%), immersion time (2-8 min) and PLA solution temperature (30-50 °C), were studied using the Design of Experiment (DOE). The analysis of the response (tear index) suggested a good fit to a quadratic model with R^2 of 0.9145. The scanning electron microscope (SEM) images of the optimized sample revealed uniform and packed dimple-like PLA morphology on the CL plus enhanced burst index, water wetting and moisture barrier properties. Due to the ease of forming sheets and their economic value, the obtained biocomposites present high potential for large scale production. This new approach shows promise for packaging applications, which require the utilization of bio-derived resources and cost effectiveness.

Keywords: cassava, poly(lactic acid), biocomposites, tear index, numerical optimization, response surface methodology

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is the most agronomically important of the cyanogenic crops, particularly as food source, and is largely grown in tropical countries. Cassava is a very rustic crop that grows well under marginal conditions, where few other crops could survive. Its flexible harvesting time and vegetative propagation via stem cutting make it an excellent food reserve.[1,2] Cassava plant is drought tolerant, can be grown in degraded soils, and is resistant to the most important diseases and pests.[3] In African countries, cassava leaves (CL) stand top in the list of leaf consumption, as cassava has become the basic vegetable and is highly valued.[4,5] In Asian countries, particularly in Malaysia, the leaves can be regarded as waste or a by-product of the harvested roots. In Southeast Asia, the growth of CL exceeded 70,000 tonnes/year, whereas only 7.0 tonnes/year of the CL were processed for production in industrial fields.

The motivation of utilizing CL is that the plant exhibits a hydrophobic waxy surface, which could form a water-repellent component to prevent the formation of a water film.[6] Furthermore, being apprehensive of the enormous amount of waste, our current work is directed towards the effort of utilizing CL for industrial applications. With the advent of technological approaches, the focus has shifted to widening the application of CL to newer applications with the aim of value adding; one of the interesting applications is as packaging material. For a long time, most common packaging materials have been dominated by the use of petroleum based plastics. However, an

increased use of synthetic packaging has led to serious ecological problems because of their total non-biodegradability. Although their complete replacement is nearly impossible to achieve, specific applications like packaging made from agricultural resources could be the future.

Our previous work has shown the potential of CL as packaging materials.[7] The CL yield sheets with good mechanical integrity and sheet making capability. Though the resulting properties of the processed leaves are promising, their mechanical strength can be improved for a viable use. It is encouraging to further enhance the CL sheets with biopolymers that possess good mechanical strength plus added hydrophobicity and good film-forming properties. One of the most promising biopolymers for such a purpose is poly(lactic acid) (PLA), as it is biodegradable, biocompatible and commercially available, with good performance characteristics as a packaging material.[8-10] In addition to its environment-friendly nature, PLA can also be used for food contact surfaces and is generally recognized as safe. Considering these characteristics and a recent decrease of the production cost due to new technology and large-scale production, PLA is becoming a good alternative as a green food packaging material, since it shows a better performance in many situations than other synthetic plastics.[11] It is interesting to synergize the advantages of agricultural waste, such as CL, with the strength of a biobased polymer, such as PLA. Since the PLA functions as a coating component, the cost of making the CL/PLA sheets can be minimized. In addition, the potential use of PLA-coated sheets in the form of trays, cartons, cups, boxes, or deli containers for packing specific products, including meat and dairy products, ready meals, beverages, snacks, dry products, frozen products, and fruits and vegetables.[12,13]

Several factors can affect the tear index of the CL/PLA biocomposite sheets: initial treatment of CL, processing temperature, PLA concentration and immersion time. Furthermore, it is troublesome to deduce the influence of these independent variables and correlate them with the properties of the resulting CL/PLA. A statistical method using the Design of Experiment (DOE) can be utilized to optimize the conditions,[14] which would produce CL/PLA with defined properties. A systematic experimental design to find the parameters that can affect tear index can be employed by utilizing the response surface methodology (RSM) procedure coupled with central composite design (CCD) and further subjected to regression analysis. RSM reduces the number of required experimental runs to achieve a statistically validated result.[15] This report focuses on the verification of factors that have significant effects on the tear index of the CL/PLA. The functional relationship between several independent variables, such as PLA concentration, immersion time and PLA solution temperature, were studied, while the interactions between variables were elucidated. The adequacies of the models were compared with experimental runs, followed by morphological characterization, bursting, water wetting and moisture absorption tests.

EXPERIMENTAL

Preparation and characterization of CL/PLA biocomposite sheets

Freshly picked Malaysian grown CL were cleaned by running tap water and then stored at a temperature of 4 °C in a refrigerator, prior to use. Their mercerization and the sheet making process have been given in detail elsewhere.[7] PLA, Nature Works™ 4031 D, was supplied by NatureWorks LLC, USA. The molecular weight (M_w) is between 195000-205000 g/mol. The PLA was mixed into methylene chloride (Merck) in various concentrations (w/v) and the polymeric solution was mechanically stirred overnight at 50 rpm, followed by heating at a predetermined temperature before immersion of CL. The initially prepared CL were immersed into the solution containing PLA for a period of time (min). The impregnated CL were then placed in a fume hood at ambient conditions to allow evaporation of the organic solvent. The PLA concentration, heating temperature and immersion time were set accordingly to the suggested experimental runs based on DOE.

The tear test was conducted using the Elmendorf Tear method (ASTM D-1922). The capacity of the pendulum used on the Elmendorf Tear Tester was 12.3 g. The sample sheets were cut according to the standard required dimensions and dried at 60 °C for 24 h before testing. A slit was made at the centre of the edge perpendicular to the tested direction. The tear index was calculated by the relation:

$$\text{Tear index} = \frac{\text{average tearing force (mN)}}{\text{average grammage } (\frac{\text{g}}{\text{m}^2})} \quad (1)$$

whereby:

$$\text{average tearing force, mN} = \frac{16 \times 9.81 \times \text{average scale reading}}{\text{number of plies}} \quad (2)$$

Bursting test was performed on a Mullen type tester according to ASTM D774. The burst index is calculated using bursting strength (kPa)/average grammage (g/m^2). Scanning electron microscope

(SEM) images were obtained using a Leo Supra 50VP. The water wetting test was performed on square CL/PLA sheets with the dimension of 1 × 1 cm. A drop of water was allowed to fall from a burette with an approximate height of 1.0 cm from the sample. The time required to wet a 1 cm distance was recorded with a stopwatch. At least three replications were done for each CL/PLA sheet. Moisture absorption test was done in triplicate on CL/PLA samples of 2 x 2 cm, placed on top of a wire mesh in five different relative humidity (RH) environments conditioned in desiccators, in accordance to ASTM E-108 standards. The RH was controlled using saturated salt solutions of LiCl (11%), KCH_3CO_2 (25%), $Mg(NO_3)_2$ (53%), NaCl (75%), and K_2SO_4 (97%). Samples were weighed at intervals of 6 hours until they reached saturation. The equilibrium moisture content at each water activity was calculated on a dry basis. The percentage of moisture absorption (%) was calculated as:

$$\text{Moisture absorption} = \frac{w_f - w_i}{w_i} \times 100\% \quad (3)$$

where w_f = final weight and w_i = initial weight.

Statistical analysis using Design of Experiment

Design Expert Software, version 6.0.6 (Stat-Ease Inc., USA), based on RSM in conjunction with CCD, was used to perform the statistical analysis and generate the regression model to analyze the correlations between CL/PLA to the response. The selected response was tear index (Y). The variables in this study included three numerical factors: that of PLA concentration (x_1), time of immersion (x_2) and temperature of PLA solution (x_3). The ranges of the independent variables and design level are shown in Table 1. The number of experimental runs determined from CCD was 20 runs, corresponding to 6 factorial points, 6 axial points and 6 centre point replications. An α value of 1 was used. The experimental sequence was randomized in order to minimize the effects of uncontrolled factors. Each response was used to develop an empirical model, which correlated the response to the variables used.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

The general quadratic equation model is given by Equation 4,[16] where Y represents the response, β_0 is the constant coefficient, β_i, β_{ii} and β_{ij} are coefficients for the linear, quadratic and interaction effects, respectively. x_i and x_j are the independent variables, and ε is the standard error. The coefficient of determination R^2 was used to evaluate the quality of the developed model, followed by the analysis of variance (ANOVA) to check the statistical significance of the model. Numerical optimization was computed by the software to give out the optimum combinations of parameters in order to fulfil the requirements as desired. The optimization was conducted for the response of tear index (Y). The ultimate goal of this optimization is to obtain the maximum response (tear index) that satisfies all the variables properties. Experimental validation was done based on the suggested runs with the highest desirability, D function.

RESULTS AND DISCUSSION

Development of the regression model equation

The complete design matrix for preparing the CL/PLA and the response data are given in Table 2. The tear index was found to range from 702.10 to 878.70 mN·m^2/g. The quadratic model was suggested, according to the sequential model sum of squares. The final empirical formula model for tear index (Y) in terms of their coded factor, after excluding the insignificant factors, is given as Equation 5:

$$Y = 868.99 - 23.00x_1 + 23.41x_2 - 62.80\,x_1^2 \quad (5)$$

The quality of the model was evaluated based on standard deviation and correlation coefficient R^2 value. The capability of the model to predict the response is translated to small standard deviation and R^2 value closer to unity. The standard deviation of Equation 5 is 21.74 with R^2 of 0.9145, respectively. This indicates that 91.5% (tear index) of the total variation was attributed to the experimental variables studied.

Table 1
Independent variables and their coded variables

Variables	Code	Coded variable levels		
		-1	0	1
Concentration, %	x_1	6	8	10
Time, min	x_2	2	5	8
Temperature, °C	x_3	30	40	50

Table 2
Experimental design matrix and responses

	Variables			
Run	x_1	x_2	x_3	Y_1
1	10.0	2	50.00	729.5
2	10.0	8	30.00	780.0
3	8.0	5	40.00	867.5
4	8.0	2	40.00	850.4
5	6.0	8	30.00	848.6
6	10.0	5	40.00	770.5
7	8.0	5	40.00	868.8
8	8.0	5	40.00	866.6
9	8.0	5	30.00	866.3
10	8.0	8	40.00	840.2
11	6.0	2	30.00	801.8
12	8.0	5	40.00	858.5
13	8.0	5	40.00	857.6
14	6.0	2	50.00	702.1
15	10.0	2	30.00	750.5
16	6.0	8	50.00	828.8
17	8.0	5	40.00	878.7
18	6.0	5	40.00	850.0
19	10.0	8	50.00	770.8
20	8.0	5	50.00	878.0

Table 3
ANOVA analysis

Source	Sum of squares	Degrees of freedom	Mean square	*F* value	Prob > *F*
Model	50568.89	9	5618.77	11.88	0.0003
x_1	5290.00	1	5290.00	11.19	0.0074
x_2	5480.28	1	5480.28	11.59	0.0067
x_3	1904.40	1	1904.40	4.03	0.0725
x_1^2	10847.13	1	10847.13	22.94	0.0007
x_2^2	2118.37	1	2118.37	4.48	0.0604
x_3^2	2.25	1	2.25	4.759E-003	0.9464
x_1x_2	1318.41	1	1318.41	2.79	0.1259
x_1x_3	996.81	1	996.81	2.11	0.1771
x_2x_3	1051.11	1	1051.11	2.22	0.1668

ANOVA analysis

ANOVA has been employed to further justify the significance of the models. The statistical significance of the quadratic model for each response is determined by the *F*-value and Prob.>*F*. The *F*-value is a measurement of the variance of data around the mean, based on the ratio of the mean square of group variance due to error. A significant model possesses a high *F*-value and Prob.>*F* less than 0.05. The ANOVA for the quadratic model of the tear index is presented in Table 3. The quadratic model of the tear index exhibits an *F*-value of 11.88 and Prob>*F* of 0.0003. x_1, x_2 and x_1^2 are the significant model terms to the response. This implies that the suggested model is significant. Based on the statistical analysis, the model is adequate enough to predict the tear index within the range of the variables.

Figure 1 shows the predicted values versus the experimental values of the responses. It can be seen that the response models show good fits to the experimental data, which reflected good predictions of the models. It is essential to check the interaction effect between the model terms based on its significant effects to the model equation. These interactions can be depicted clearly by plotting both variables on a 2D plot and 3D surface plot. Since the model has more than two variables, only the targeted variables were varied while the others were held constant. Based on the *F*-values, PLA concentration gives the

greatest impact on the response. The temperature factor is set at low level (-1.0).

The 2D and 3D plots presented in Figure 2a and b, respectively, show the interaction of the PLA concentration and immersion time towards the tear index of the CL/PLA. It can be observed from the 2D plot that the maximum tear index of the CL/PLA is provided at the lower limit region of PLA concentration (7 to 8%) for both immersion times (2 and 8 min). An immersion time of 8 min led to a higher tear index for the CL/PLA than that of 2 min at the same PLA concentration. It has been shown by the numerical optimization that it is possible to achieve the maximum tear index with the shortest immersion time. Upon another close examination of the contour plot (Fig. 2b), it can be noted that the process may be slightly more sensitive to changes in PLA concentration than to changes in immersion time. An increase in PLA concentration increases the tear index up to an optimized level and decreases thereafter. An insufficient amount of PLA could cause the surface to be dominated by the solvent rich component, thereby leaving behind a significant portion of bare leaf (uncoated surfaces). The 3D plot also implies that high PLA concentration has an adverse effect on the mechanical strength of the biocomposite sheets. Upon higher impregnation concentration (10%) the tear index drops severely. This poor resistance to crack propagation is due to the difference in stiffness of PLA and CL, which generates an additional internal stress. Furthermore, the weight of the PLA component contributes to the lowering of the tear index due to the specific weight relation of the testing, as can be seen in Equation 1.

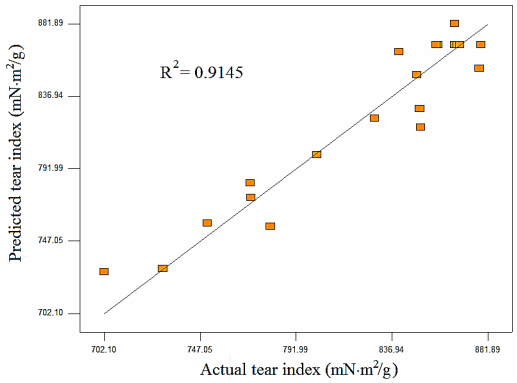

Figure 1: Comparison between the actual and experimental values of tear index

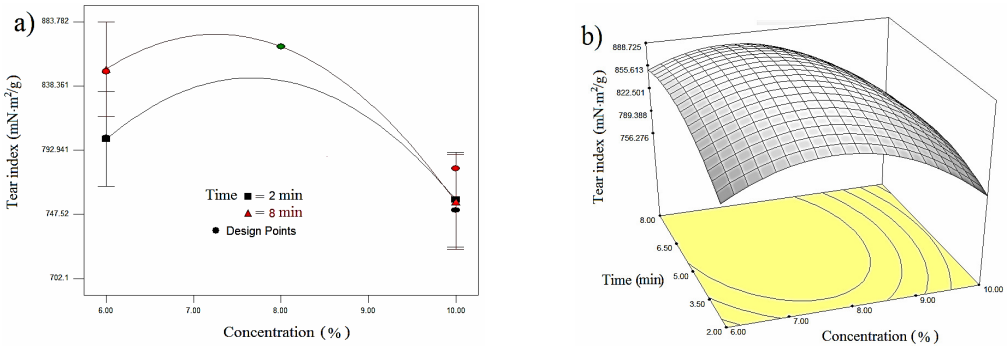

Figure 2: a) 2D and b) 3D response surface plot of tear index

Process optimization

Table 4 gives the constraints of each variable for the numerical optimization. The concentration factor was set to be in range since it is the most significant factor. The immersion time was set to minimum in order to minimize the preparation time and the temperature was also set to minimum, since the term was not significant based on the ANOVA. Table 5 exhibits the three possible runs, which fulfilled all the specified constraints of Table 4 to obtain the optimum value for the tear index. All the runs in Table 5 give high *D* values. The optimum conditions in any of the three given runs can be chosen for

further validation. Runs no. 1 and 2 in Table 5 obtained the highest *D*. Both runs could be used to validate the actual performance of the final product. In this case, Run no. 1 (7.66% of PLA, immersion time of 2 min and PLA solution temperature of 30 °C) was selected. For further validation of the developed model, the experimental runs were carried out with the conditions as stated in Table 5, Run no. 1. The experiments were repeated three times to ensure the validity of the results. Table 6 shows the three repeated runs for the model validation. The accuracy of the equations derived from ANOVA can be evaluated by calculating the percentage of errors between the predicted and the experimental response of tear index. The results confirm the predictability of the ANOVA model for the response under the experimental conditions. The errors of predicted and experimental values of the tear index were less than 5%. It can be justified by the developed model and process optimization that the tear index of the CL/PLA can be optimized using all the possible runs.

Table 4
Constraints of each variable for the numerical optimization of tear index

Type of variable	Goal	Lower limit	Upper limit
Concentration, %	in range	6	10
Time, min	minimum	2	8
Temperature, °C	minimum	30	50
Tear index, $mN \cdot m^2/g$	maximize	702.10	878.70

Table 5
Optimum conditions to obtain the maximum tear index

Run no.	Numerical factors			Tear index, $mN \cdot m^2/g$	Desirability, *D*
	Concentration, %	Time, min	Temperature, °C		
1	7.66	2.00	30	844.01	0.930
2	7.62	2.00	30	843.99	0.930
3	7.66	2.14	30	847.20	0.929

Table 6
Results of validated experiments conducted under optimum conditions as obtained from DOE

Run no.	Numerical factors			Predicted tear index, $mN \cdot m^2/g$	Experimental tear index, $mN \cdot m^2/g$	Error, %
	Concentration, %	Time, min	Temperature, °C			
1	7.66	2.00	30	844.01	861.72	2.06
2	7.66	2.00	30	844.01	825.86	2.15
3	7.66	2.00	30	844.01	816.33	3.28

Characterization of optimized biocomposite sheets

SEM

Figure 3a depicts the SEM image of uncoated CL. Figure 3b shows the obtained CL/PLA using the suggested run from the DOE (Run no. 1). The optimized surface exhibits a much different morphology than that of the uncoated CL. A uniform PLA surface coating can be seen. The entire surface of the processed leaves has been coated with PLA by solution dipping, no exposed surface is observed. This easy to process and high mechanical strength biopolymer provides the reinforcement needed for utilizing the CL sheets. Higher magnification on the surface (Fig. 3c) revealed a uniform internal dimple-like pattern, indicating kinetically trapped morphology as a result of solvent evaporation.[17] These micron sized pores can enhance the hydrophobicity of the substrate surface (CL). Furthermore, these dimples were closely packed with average internal diameters of 1.7 μm (Fig. 3d).

Burst index, wetting time and moisture absorption

Burst index represents how much pressure the sheet can tolerate before rupture. The burst index of the neat processed CL was of 0.7 ± 0.2 $kPa \cdot m^2/g$. The optimized impregnated CL/PLA achieved a burst index up to 1.4 ± 0.3 $kPa \cdot m^2/g$. This two-fold increment is highly due to the PLA component, which provides significant polymeric

elasticity to the composite during the bursting test. The water wetting time of the optimized CL/PLA revealed a highly hydrophobic surface. The CL/PLA took more than 300 min to wet compared to that of neat CL (15 min). This might be due to the formation of inverted PLA microspheres, as seen in the SEM of Figure 3, which provide high mismatch of surface tension. This proved that the suggested run is suitable for increasing the water resistance of CL. Figure 4 presents the moisture uptake of the neat processed CL and optimized CL/PLA. It can be seen that the CL show progressive increment of moisture absorption with increasing RH. Increasing humidity conditions increase the sheets moisture uptake.

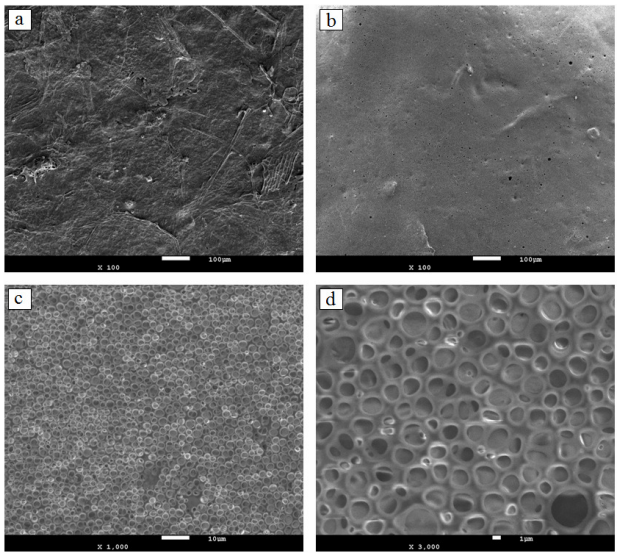

Figure 3: SEM images of a) neat CL sheet, b) optimized CL/PLA, c) higher magnification of optimized CL/PLA (1000 ×) and d) higher magnification of optimized CL/PLA (3000 ×)

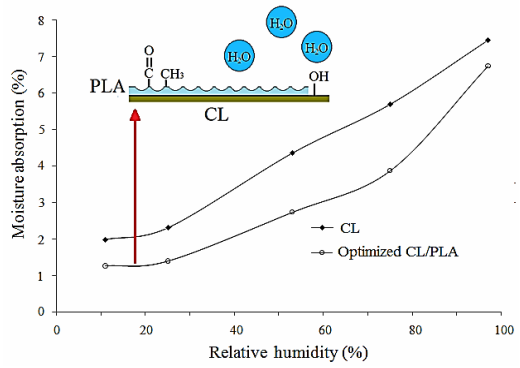

Figure 4: Moisture absorption of optimized CL/PLA biocomposite sheet with increasing RH

The optimized CL/PLA exhibit a quite similar increasing moisture absorption trend with humid surroundings, but with much lower magnitude compared to that of its uncoated counterpart. This reduction can be explained by the hydrophobic features of the PLA component that surrounds the CL surfaces. The manner in which the PLA is bounded on the surface enables it to act as a barrier and prevent the contact between water molecules and CL surface hydroxyl groups.[18] The insert of Figure 4 depicts the interactions between CL and water molecules of the optimized CL/PLA. Such modification could reduce the degree of water uptake, which in turn will preserve the functional, structural and mechanical properties of the packaging sheets.

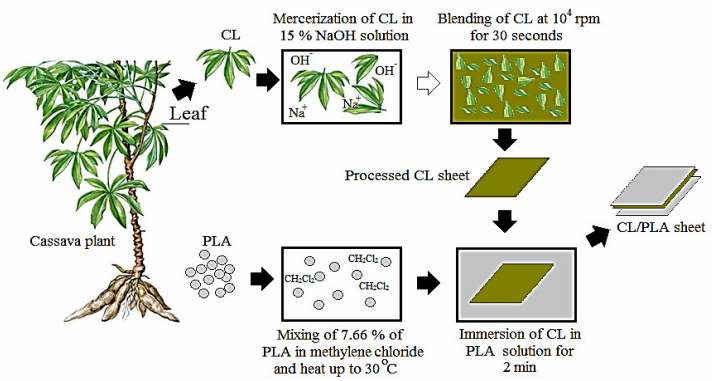

Figure 5: Process of making CL/PLA sheet

CONCLUSION

The effects of poly(lactic acid) concentration, immersion time and solution temperature on the tear index of cassava leaves/poly(lactic acid) biocomposite sheets were successfully studied using response surface methodology equipped with central composite design. A quadratic model was given to correlate the process variables to the response. The design of experiment revealed that the poly(lactic acid) concentration had the most significant influence on the response. Optimum process conditions were obtained at PLA of 7.66%, immersion time of 2 min and solution temperature of 30 °C, which resulted in a tear index of 844.005 $mN \cdot m^2/g$. Scanning electron microscope images of the optimized biocomposite sheet revealed uniform coating of poly(lactic acid) with dimple-like morphology. The optimized process of making the cassava leaf/poly(lactic acid) biocomposite sheets can be summarized by the illustration of Figure 5.

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