Microwave Release of Pectin from Orange Peel Albedo Using a Closed Vessel Reactor System

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Pectin was extracted from Moro blood orange in a closed vessel reactor heated with microwave irradiation. Time of heating was either 2 minutes at 110 °C or 210 minutes at 75 °C at pH values of 1.7 to 2.8. A run at 75 °C and at pH 1.7 with resistive heating was additionally performed to simulate industrial process conditions. The highest recovery of pectin [anhydrogalacturonic acid (AGA) of 3.44 milligram per milliliter] was observed at 110 °C and pH 1.7 with microwave heating but the degree of methylation (DM) under these conditions was the lowest at 50.3 percent. A DM of 72.7 percent was observed at 110 °C and pH 2.8, but the pectin concentration was lower at an AGA value of 2.08 mg per milliliter. At pH 2.2 both the DM and AGA values were intermediate between the other two reaction conditions suggesting that lower pH improves yield but at the expense of a lower DM value. Molecular weight changes were observed with the various heating and pH values, but the largest effects were on AGA recovery and DM. The data suggests that by careful selection of pH and temperature, acceptable yields of pectin using microwave heating can be obtained while preserving a significant portion of the DM.

A major component of citrus peel is pectin, and one approach toward the use of a large fraction of citrus byproducts is to extract spent peel and then to modify the pectin component prior to use. In fact, the most common source of pectin for food and pharmaceutical applications is from citrus peel, primarily lemon—but lime, orange, and grapefruit may also be used (May, 1990). Applications are typically limited to food and pharmaceuticals due to the high cost of extracting and purifying pectin, and production volumes are relatively low. To gain entrance in larger volume industrial markets required to use the large quantities of spent citrus peel, more cost effective extraction and purification procedures are required.

Pectin is a complex polysaccharide composed of at least five different sugar moieties where 80% to 90% of its dry weight is anhydrogalacturonic acid (AGA). The majority of the AGA is found in homogalacturonan (HG) regions of pectin as unbranched polymers of AGA in which a variable proportion of the AGA residues contain a methyl ester at their C6 position (Ridley et al., 2001; Vincken et al., 2003). Pectin's functional properties and reactivity toward calcium and other cations are largely dependent on the amount of methylated galacturonic acid subunits and their distribution pattern within the HG stretches (Powell et al., 1982; Willats et al., 2001). Pectins, with lower amounts of methylated galacturonic acid and more reactive to calcium ion, have been shown to require relatively harsh extraction conditions (pH 1.7 for 3.5 h at 75 °C) (Joye and Luzio, 2000). Preserving high DM during extraction is important for many commercial applications, since gel time and ionic crosslinking with divalent cations such as calcium ion is dependent on DM (Garnier et al., 1993; Morris et al., 1982).

Extraction of pectin may be done either under acidic or basic conditions, but in a typical commercial process, acid is used to preserve molecular weight and to minimize loss of ester functionality (May, 1990). In acid extraction, plant material is treated with acid (pH between 1.5 to 2.0) at temperatures between 70 and 90 °C for a time sufficient to solubilize the pectin which may be bound to the insoluble material such as cellulose (Fishman et al., 2000; Joye and Luzio, 2000). Extracted juice containing the pectin fraction is then separated from the reaction mixture by filtration. Pectin is typically precipitated from the extracted juice by alcohol precipitation using isopropyl alcohol (IPA) and then dried and milled to a desired particle size.

Recent work on a laboratory scale has shown that pectin, flash (rapidly) extracted from orange albedo by microwave heating under pressure has increased molar mass, size and intrinsic viscosity when compared with pectin extracted by resistive heating techniques using short reaction times (Fishman et al., 2000). This work was done with a multi-mode microwave cavity system, where several samples can be heated together in one large chamber. This type of multi-mode heating can be problematic if the microwave field in the chamber is non-uniform. Newer designs are now available with single mode cavities where the uniformity of the microwave fields is controlled (Ferguson, 2003). Single mode systems propagate only one mode of microwave energy in one sample location which eliminates positional sensitivity. The work reported here was done using a single-mode cavity microwave system. In addition, rapid cooling is available in this single-mode to effectively stop the hydrolysis reaction and provide precise control of the reaction times.

Material and Methods

MATERIALS. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Moro blood oranges were hand picked in Feb. 2008 from tree 12-6 located at the Florida Department of Agriculture (3027 Lake Alfred Road, Winter Haven, FL).

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SAMPLE PREPARATION AND EXTRACTION. Immediately after harvesting, the flavedo from Moro blood oranges was removed using a potato peeler and the albedo was then removed by hand. Samples of albedo were not prewashed, and the albedo was chopped and immediately frozen for processing at a later date. Pectin from the albedo was extracted using a modified microwave acid extraction procedure previously described (Fishman et al., 2000). Samples were extracted either at pH 1.7, 2.2, or 2.8. Following pH adjustment using acid, the albedo suspension was irradiated at a frequency of 2455 MHZ with 50 W of power for samples heated to 110 °C and at 2 W of power for samples heated to 75 °C. A slurried sample at the specified pH (7mL containing 14.0 mg/mL dry peel solids) was placed in each of six microwave-transparent pressure vessels. Samples were heated in a CEM Focused Microwave Synthesis System, Model Discover (CEM Corp. Matthews, NC). Control of pressure, temperature and microwave power was performed after inputting empirically-determined control values into the CEM Synergy™ software. Microwave heating times were for 210 min at 75 °C and for 2 min at 110 °C with mixing. In addition, a control extraction was performed by heating for 210 min at 75 °C using a resistive heater with mixing. After heating, the samples were cooled in the instrument to room temperature in approximately 2 min (see Fig. 1) and then centrifuged at 3000 × g. The supernatant was removed and precipitated with cold IPA at a concentration of 70% for 60 min, separated from the mother liquor by centrifugation (10,000 × g). The wash and centrifugation repeated two more times with cold 70% IPA. The samples were then dried in vacuum at 50 °C overnight and stored in a refrigerator until analyzed. Samples were rehydrated in 5.0 mL of deionized water with 0.002% lithium azide as a preservative by stirring for a minimum of 24 h at room temperature. Four or six replicates were performed for each set of extraction conditions.

MOLECULAR SIZE AND COMPOSITIONAL ANALYSIS. Anhydrogalacturonate content (% AGA) was determined by the dimethylphenol colorimetric method (Scott, 1979) as modified into a microplate procedure (Luzio, 2004). All yields were computed from the ratio of the AGA values obtained after extraction in mg/mL together with corresponding dilution values and the initial amount of dry peel solids present in the reactor tube of 14.0 mg/mL. The calculation takes into account the initial volume of peel which was 7 mL while the final volume used for dissolving the pectin was 5.0 mL. Degree of methyl esterification (% DM) was determined by a previously developed HPLC method (Voragen et al., 1986). Molecular size analysis was performed by multiangle laser light scattering size exclusion chromatography (MALLs-SEC) as previously described (Luzio, 2006).

Results and Discussion

In an acid extraction process, citrus peel is typically treated with acid, pH of 1.5 to 2.0, at temperatures between 70 and 90 °C for a time sufficient to solubilize the pectin which may be bound to the insoluble material such as cellulose (May, 1990). Control of temperature and heating time is important since these conditions affect the amount of pectin extracted, the DM and the molecular weight of the pectin. To test thermal control of the extraction conditions, a sample of Moro blood orange peel slurry, adjusted to pH 1.7, was placed in the CEM microwave reactor and heated for 2 min at 110 °C. The heating and cooling curve is shown in Figure 1, and the specific reaction conditions are noted in the legend. Finding the appropriate reactor condi-
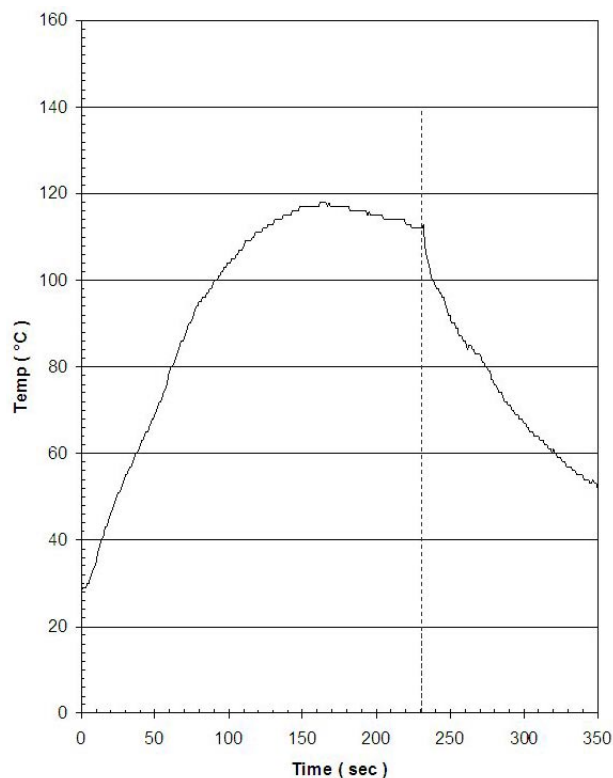

Fig. 1. Representative heating and cooling curve for microwave extraction of albedo for target heating to 110 °C for 2 min showing actual reactor temp (——) in °C. In this particular run microwave power was limited to 50 W. Vertical dashed line (– – – –) at 230 s indicates initiation of the compressed air cooling phase and the termination of microwave irradiation.

tions was achieved by empirical manipulation of the reactor power settings, maximum pressure and temperature settings, until reproducible control of these parameters was observed. As shown, the temperature reached the 110 °C target within 110 s and it held approximately that temperature for 2 min until 230 s, at which time the cooling cycle was initiated as illustrated by the vertical dotted line. The cooling cycle was completed within 2 min, which effectively terminated the extraction process. These data demonstrate that the temperature and heating time interval can be carefully controlled by proper instrument settings and that test samples are not exposed to excessive thermal spikes or long delays in cooling which could affect the extraction results. Temperature and pressure curves were monitored for every extraction to insure that conditions stayed within the set limits.

A sample prepared under the same set of conditions as shown in Figure 1 was subjected to MALLS-SEC analysis. The molecular weight obtained from MALLS, together with the refractive index (RI) data, are shown in Figure 2. The MALLS exhibited a maximum molecular weight at the void volume of 20 mL that indicated a high molecular pectin component in excess of 1×10^7. The concentration of this high molecular weight pectin was very low at the void as indicated by the negligible response from the RI detector. The elution profile of the RI detector (concentration detector) was bimodal with two maxima. One maximum from the RI was in the medium molecular weight region (28.5 mL elution volume) and exhibited a molecular weight of 1.21×10^5 ($\pm 0.007 \times 10^5$) and the second maximum was in the low molecular weight region (34.5 mL elution volume) with a molecular weight of 3.53
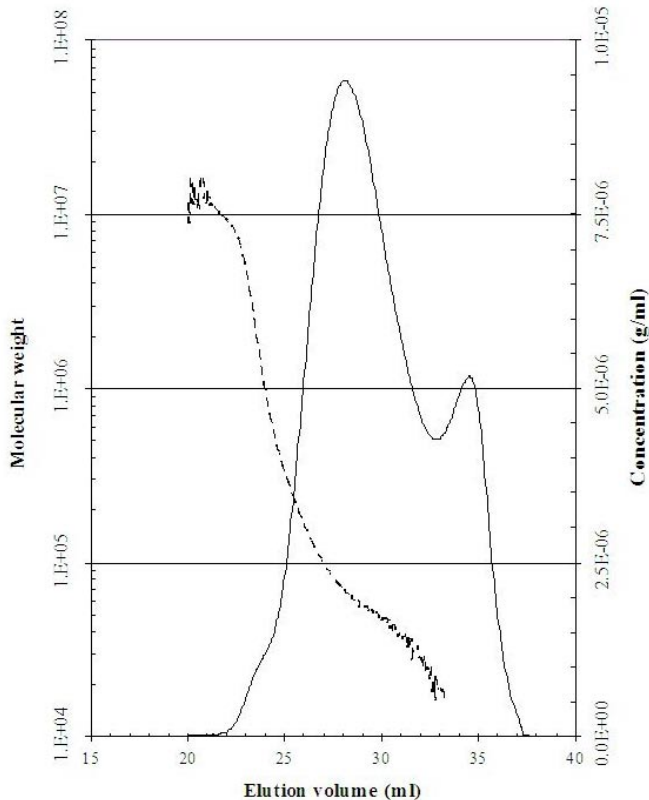
Fig. 2. Molecular weight determined by MALLS (– – – –) and concentration value obtained from the RI detector (——) for orange peel extracted at 110 °C for 2 min using microwave irradiation.
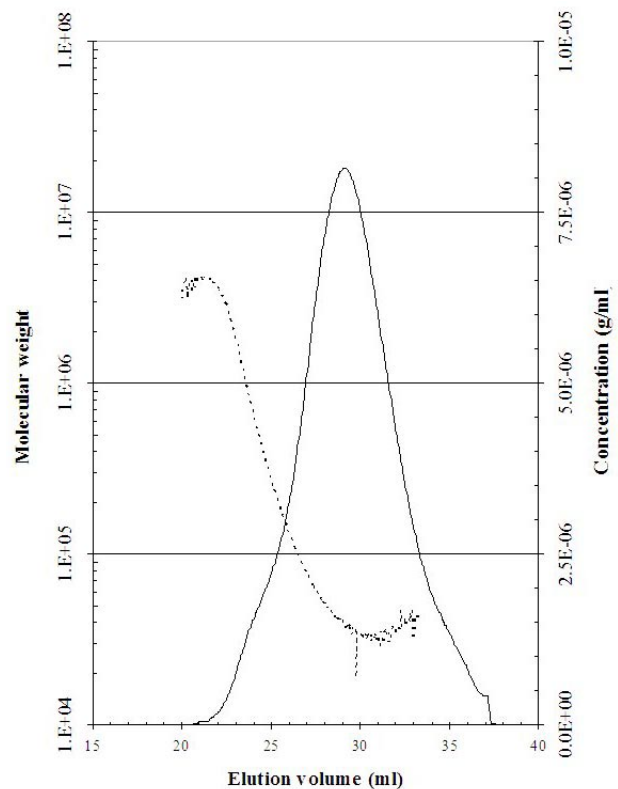

Fig.3. Molecular weight determined by MALLS (– – – –) and concentration value obtained from the RI detector (——) for orange peel extracted at 75 °C for 210 min using resistive heating.

$\times 10^5$ ($\pm 0.55 \times 10^5$). This can be compared to the data observed with the control extraction where a duplicate sample of Moro blood orange peel was extracted at the same pH of 1.7 but for 210 min at 75 °C using electrical resistance as the heat source instead of microwave heating. The elution profile data for this conventional acid extraction is shown in Figure 3. The MALLS detector exhibited a lower molecular weight of 4×10^6 at the void volume (as compared to the microwave extraction) and the profile of the RI detector showed a relatively symmetrical peak shape with a single maximum from the RI detector at 29.0 mL elution volume with a molecular weight of 5.46×10^4 ($\pm 0.105 \times 10^4$). Thus, differences in reaction conditions, such as the heat source and time of heating, appeared to have an effect on the molecular properties of the extracted pectins. This difference is important because molecular weight and polydispersity should have an effect on the rheological properties of the pectin solutions in a given application.

The differences in extraction conditions can also be observed in Fig. 4 where the radius of the molecule (Rw) is plotted as a function of molecular weight for the two different types of extractions. As shown, the Rw for the resistive heat extraction resulted in no significant change in radius size from 30 nm to 35 nm in a molecular weight range of 1×10^5 to 4×10^6. Pectins, in excess of 4×10^6 molecular weight, were not observed in this particular extraction using resistive heat. Similarly, the microwave heated sample showed no significant change in Rw, which again ranged from 30 to 35 nm, with increasing molecular weight and this sample contained pectins with molecular weights extending up to 1×10^7. Unlike variations in molecular weight with the type

of heating source, there were no significant differences observed for the radius values.

The average values of the different parameter runs were determined and this data is shown in Table 1. Extraction conditions are given in the first four columns and corresponding analysis values for RI (total polysaccharide), Mw, AGA (total pectin), DM and yield are also given. Mw here represents a weight average molecular weight, and is typically higher for pectins than the values discussed above corresponding to values at the RI maximum, which is closer to a number average molecular weight or Mn. For the Mw values the maximum molecular weight was observed at pH 2.2 with a molecular weight of 687,000, and the lowest molecular weight of 183,000 was at pH 1.7 with microwave heating for 210 min at 75 °C. The control extraction, at pH 1.7 and heating for 210 min at 75 °C using resistive heating showed a molecular weight of 255,000. All of the rapid 2-min reaction time samples at 110 °C heated by microwaves had higher Mw than the control with resistive heat at 75 °C. This indicates that microwave heating can be used as a heat source and still maintain high molecular weight which is important for many applications. There were no observable differences in Rw and all values were within one standard of the Rw of 35.5 nm (± 9.3 nm) for the citrus peel extracted with resistive heat at pH 1.7. Again this indicates that microwave heat is an acceptable substitute resistive heat while maintaining desirable molecular properties such as molecular radius.

For DM values, the control extractions using a resistive heat source had a DM of 72.4% which is typical for commercial rapid-set pectin. A similar value of 72.7% was observed for the pH 2.8
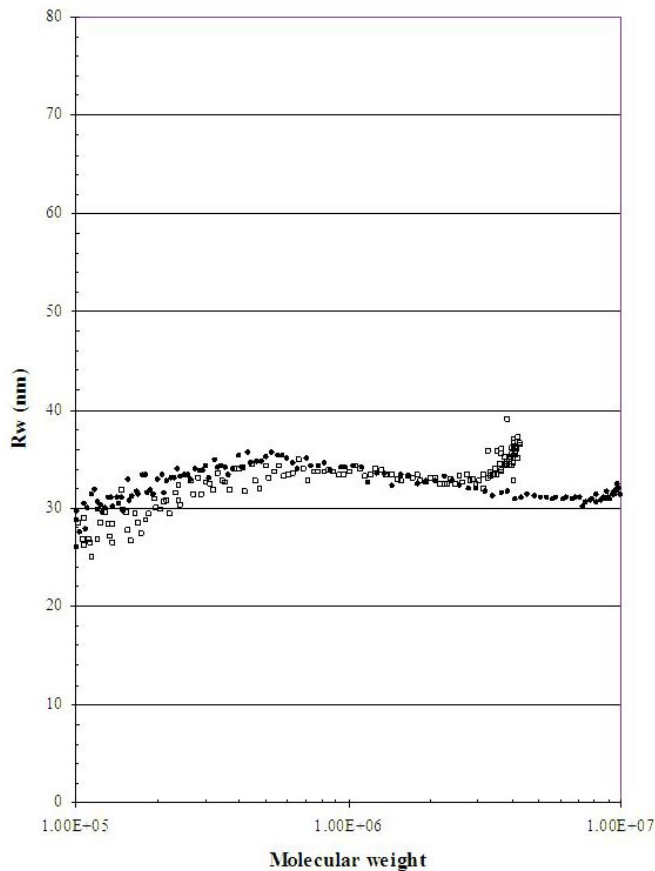
Fig. 4. Root mean square radius Rw as a function of molecular weight for albedo extracted at pH 1.7 using microwave energy at 110 °C for 2 min (●) and resistive heating at 75 °C for 210 min (□).

rapid microwave heating extractions at 110 °C. At the lower pH values of 2.2 and 1.7 with rapid microwave heating at 110 °C using, the DM values were 64.6 and 50.3 % respectively. These data indicate that low pH combined with microwave heating at high temperatures is deleterious to DM. This loss in DM as compared to the control with resistive heat was also observed for the microwave heating at pH 1.7 for 210 min at 75 °C. The only variable that was different in this low temperature experiment with microwaves as compared to the control reaction was the type of heat source employed. As noted previously preserving a high DM during extraction is important. Additional experiments were performed using pure pectins adjusted to the same pH of 1.7,

and loss in DM with microwave heating at 110 °C for 2 min was not observed (data not shown). The loss in DM under conditions with citrus peel extractions appears to be a milieu effect requiring components present in the citrus peel together with microwave energy for loss in DM to occur. Additional experiments will need to be performed for a better understanding of this phenomenon. It is worth noting, there have been previous reports in the literature pertaining to the catalysis of ester hydrolysis in the presence of microwave energy (Carta et al., 2002; Dayal et al., 1991). Loss of pectin DE was not apparent using a multi-mode microwave cavity system (Fishman et al., 2000), but it was observed here using a single-mode microwave cavity system. Perhaps the design of the microwave heating system may have some influence on this observed difference.

Another important value that was measured is the quantity of pectin extracted, and this could be indicated by two independent assays, RI and AGA. As defined here, the RI is the total quantity of polysaccharide observed by the total integration of the RI curve as determined from a SEC\MALLs experiment. RI is a non-selective detector and will respond to all polysaccharides and other solutes that may co-elute with pectin during the SEC/MALLs experiment. For this reason the RI value may be larger or equal to the value for the pectin obtained by AGA analysis, which is specific only for pectin, and should be more accurate for determining pectin recovery. All yields were computed from the ratio of the AGA values obtained after extraction and the initial amount of dry peel solids present in the reactor tube. As shown in Table 1, the control reaction with resistive heat had an AGA value of 1.80 mg/mL for a yield of 8.76% based on the initial dry solids of unwashed wet peel extracted. This value was lower than the highest value of 3.44 mg/mL with the pH 1.7 rapid microwave heating at 110 °C, where the yield was 17.0%. Lower yields and AGA values were observed with the pH 2.2 and pH 2.8 reactions with microwave heating where the yields were 14.9% and 10.3% respectively. The inverse relationship using microwave heating between pH and percent yield is consistent with results typically observed with resistive heating (Joye et al., 2000). In particular, the yield of 10.3% using microwave heating for 2 min at pH 2.8 and 110 °C was comparable to the value of 8.76% obtained after 3.5 h of heating at pH 1.7 at 75 °C in the control. This data suggests that very rapid extractions of pectin with acceptable yields can be achieved at relatively high pH values and high temperatures of extraction. Such reaction conditions could aid in developing a more cost effective extraction process since much lower amounts of acid would need to be added to an extraction mixture as compared with resistive heat extraction procedures. In addition, under these specific pH, time and microwave conditions the

Table 1. AGA, RI, DM, yield and Mw values for pectins extracted from Moro blood orange albedo under various heating and pH conditions. Mw represents weight average molecular weight and does not take into consideration the relative number of molecules present at a particular molecular weight. Rw represents the radius of the molecules. Each individual sample was analyzed in duplicate and the values average for a particular run. Either 4 or 6 samples were extracted for each run. Values in parenthesis represent standard deviations of the values observed for the total runs performed. In temperature column M denotes microwave heating and R denotes resistive heating using a heating block.

pH	Runs (no.)	Heat time (M)	Temp (°C)	Mw (× 10⁻⁵)	Rw (nm)	DM (%)	RI (mg/mL)	AGA (mg/mL)	Yield (%)
1.7	4	2.0	110 M	3.91 (0.36)	34.3 (2.2)	50.3 (2.6)	3.45 (0.25)	3.44 (0.47)	17.0 (3.4)
2.2	6	2.0	110 M	6.87 (0.41)	38.7 (1.5)	64.6 (1.8)	3.78 (0.34)	2.92 (0.10)	14.9 (0.7)
2.8	6	2.0	110 M	3.50 (0.66)	30.6 (8.8)	72.7 (1.8)	2.92 (0.24)	2.08 (0.19)	10.3 (1.3)
1.7	4	210	75 M	1.83 (0.21)	36.6 (7.8)	56.1 (2.8)	2.73 (0.43)	2.15 (0.14)	10.2 (1.0)
1.7	4	210	75 R	2.55 (0.29)	35.5 (9.3)	72.4 (2.4)	2.65 (0.16)	1.80 (0.14)	8.76 (1.0)

DM is preserved as noted previously, which is also important for process considerations. Finally it is worth noting, that all of the RI values were equal to or greater than the AGA values, which indicates that some impurities were present in the pectins after separation from the peel material. Additional experiments are required to determine the exact nature of these impurities present following extraction and purification of the pectins.

Conclusions

MW differences were observed with microwave heating as compared to resistance heating and by adjusting pH values, but perhaps the largest effects were on AGA recovery and DM. The data suggests that by careful selection of pH and temperature, acceptable yields of pectin can be obtained while preserving a significant portion of the DM. It would appear that the presence of microwaves as a heat source could also catalyze a deesterification reaction during citrus peel extraction at low pH. If preservation of DM is important in the process then careful control of microwave reaction conditions and pH has to be employed for large scale extractions.

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