

Performance Comparison of Single-Mode and Batch Microwave Reactors Using Curcuminoid Synthesis

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ABSTRACT

Microwave chemistry is a current and quickly developing area of chemistry. Just in the last five years the *Journal of Chemical Education*, an American Chemical Society publication that focuses on bringing innovations in chemistry into classrooms, nearly doubled their amount of published research targeting laboratory incorporation of microwave experiments. Microwave synthesis offers multiple advantages, such as reduced reaction times, alternative regioselectivity, and fast screening of reaction conditions. The reactions are usually carried out using microwave reactors that differ in their fundamental setup of microwave experiments. Due to the high cost of these instruments side-by-side studies of their performance towards synthesis are rare which limits knowledge of end users on best instrument for their needs. In this project, we undertook a comparison study to evaluate performance of two major brands of microwave reactors using synthesis of curcumin and two curcumin-related molecules as modeled reactions. In addition, we investigated environmental impact of our solvent-free methodology towards curcuminoids versus traditional solvent-based synthesis using EATOS software.

INTRODUCTION

In the past 150 years, the U.S. chemical industry has significantly impacted the national and global economy. According to the U.S. Environmental Protection Agency, chemical manufacturers reported producing or importing 74 billion pounds of substances per day in 2005 [1]. Data included substances used in industrial processes and did not include fuels, pesticides, pharmaceuticals, or food products. The chemical industry provides many products with many beneficial uses however they can also have negative impacts on the environment and human health.

The spice turmeric contains a mixture of natural compounds including the chemical curcumin. Curcumin and analog curcuminoids demonstrate medicinal properties of interest including anti-inflammatory and anti-cancer properties. The FDA considers turmeric and curcumin as safe products and the two are commonly sold as dietary supplements. In 2019 the worldwide market for curcumin was approximately worth \$130 million and projected to be growing [2]. In 2015 54% of the curcumin market was comprised of pharmaceutical grade curcumin. Curcumin isolated from natural sources such as from the roots of the *Curcuma longa* plant typically consists of mixtures of curcumin and curcuminoids. This mixture cannot be used for biological performances due to impurities. In our continuous efforts to develop a green industrial methodology towards obtaining structurally diverse curcuminoids we developed a solvent-free methodology that utilizes microwave energy to obtain these valuable compounds [3].

To minimize the negative impacts of chemical products and processes the concept of Green Chemistry was developed in the late 1990's. The Environmental Protection Agency defines green chemistry as the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. The Pollution Prevention Act of 1990 promoted the idea of preventing the generation of pollution in the first place [4]. This directly ties into green chemistry which focuses on source reduction at a molecular level as opposed to clearing existing pollution in an effort to keep hazardous materials out of the environment altogether. A method can be defined as "green" if it aims to incorporate one or more of the 12 designated principles of Green Chemistry that include waste prevention as well as the use of safer solvents and reaction conditions [5]. The principles of green chemistry have a wide range of application including laboratory, industrial, agricultural, pharmaceutical, and classroom settings. In our current study we explore the use of a solvent-free microwave synthesis to decrease hazardous waste and provide a better heat transfer.

Use of microwave reactor in organic synthesis is based on the availability of a high and stable electric field [6]. In microwaves, the heat is created in the interior of the reaction mixture (Figure 1a). During conventional heating, heat is transferred through the wall using a heating media, e.g. oil bath (Figure 1b). The efficient internal

heat transfer minimizes the effect of the wall retardation of potential for the seed formation and increases potential for superheating. The superheating effect is found to be important in the equilibrium reactions producing water as byproduct [7]. The observed drastically positive impact on products' production is largely attributed to the transfer of water from reaction mixture into gaseous phase under the conditions of the synthesis [8].

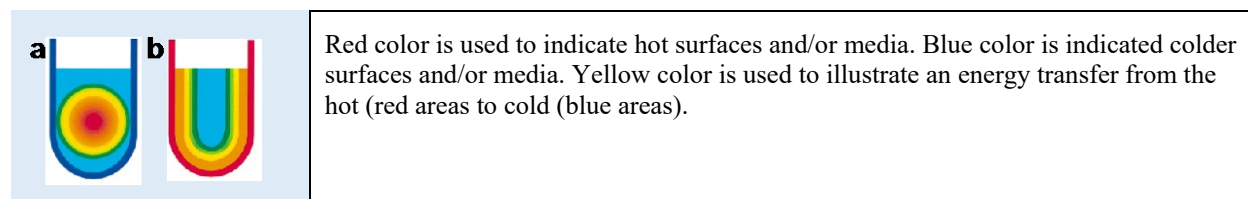


Figure 1. *In situ* heating a) microwave; b) conventional. Image adapted from (Loupe, 2002).

The microwave synthesis, although originally was performed in domestic microwaves is no longer performed that way. Domestic microwaves offer no safety measures or a control over the reactions. In addition, stirring of a reaction media or pressure monitoring during synthesis is nearly impossible. The alternative to domestic microwaves are known as microwave reactors or digestors. The price of these instruments range from \$25,000 to \$80,000 based on the manufacturers' quotes. The main suppliers of microwave reactors that were reviewed in this project are Biotage® and CEM Corporation. The first company product is known as Initiator+, fourth generation microwave synthesizer, is shown on Figure 2.

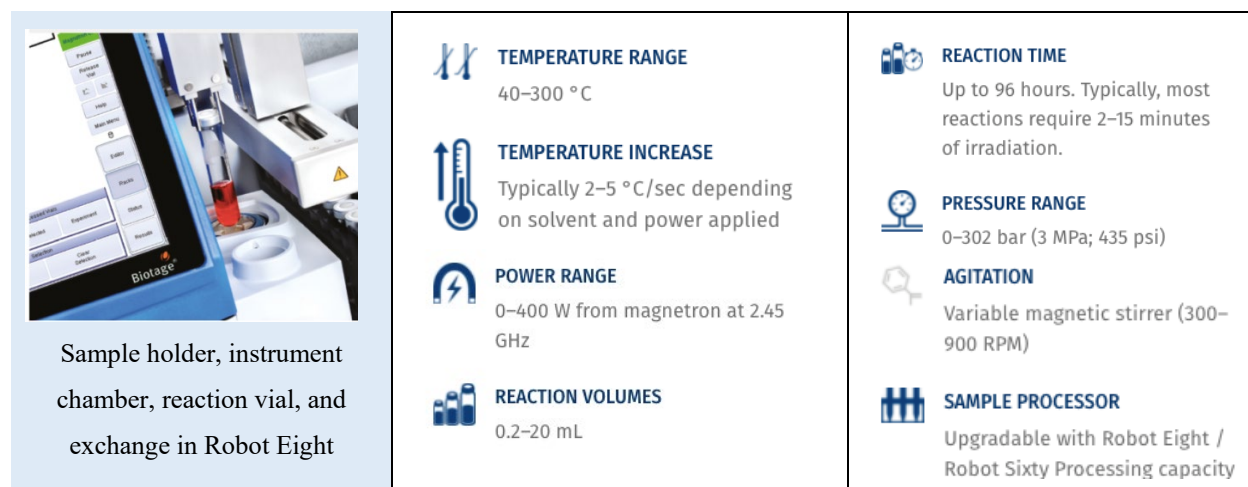


Figure 2. Biotage(R) Initiator+ and its specification. Adapted from manufacturers' site at <https://www.biotage.com/initiator-microwave-synthesis?p=specifications>, accessed May 5 2021.

The main advantage of this type of reactor is superior control over the experiment as instrument monitors both temperature and pressure during individual sample synthesis. In addition, the closed vessel setup prevents solvent evaporation during synthesis reducing safety concerns and negative environmental impact. Since each vial is sealed inside the cavity of the instrument while exposed to microwave energy the possibility of physical injuries to the researcher due to breakage of over pressurized vial is eliminated. The main disadvantage of the instrument is low output. Each experiment is run in a sequence, which not only includes the time required to run the experiment but also to cool it. On average a reaction cooling takes about a third of the reaction time which further slows down the data collection. The manufacturer offers an addition of Robot Eight or Robot Sixty which increases processing capacity to eight or sixty samples respectively. While this addition does allow setup of multiple experiments by the user it does not solve the issue of sequential running of individual syntheses. In addition, the manufacturer specified that reactions can be carried out only in microwave vials produced by Biotage® to ensure warranty and safe operation. The individual vial and cap range in price from \$1 to \$3 and only allow one use. According to the manual, single use prevents users from unintentionally micro-damaging vials by scratching the glass surface that can lead to the generation of pressure point during synthesis resulting in vial breakage and compromising instrument operation.

The second major manufacturer of microwave reactors is CEM Corporation that produces MARS 5 Digestion Microwave System pictured on Figure 3.



Temperature range 0 – 180 °C	Reaction volumes 0 – 20 mL
Power Range 0 – 1200 W 2.45 GHz	Pressure range 0 – 200 psi

Figure 3. CEM MARS 5 digestion microwave system and its specification. Information adapted from manufacturers' instrument manual.

The main advantage of this type of reactor is the ability to run multiple samples at once. Samples are held in reactions vessels which sit on a turntable that can rotate in either a continuous or alternating mode to ensure heating is distributed evenly through each sample. Another advantage is the variety of vessels that can be used with the system that vary based on volume as well as pressure and temperature ranges. There are several safeguards in place to minimize the chances of materials exploding inside the reactor. Each vessel fits into a Kevlar sleeve and the microwave contains a sensor that will cut power to the microwave if a sudden change in pressure is detected. Further safety measures include a fluoropolymer-coated microwave cavity resistant to acids and bases, cavity exhaust fans and tubing to vent fumes, and an interlocking system to prevent microwave emissions while the reactor is open. The manual also suggests 30 minutes of cooling after digestion for safety concerns. A possible disadvantage to the system is the lack of monitoring of each vessel. The reactor uses a control vessel in order to monitor the temperature and pressure of a single sample. This vessel contains a pressure sealed glass well that allows for the temperature sensor to be lowered into the sample besides this piece the control vessel is the exact same as other vessels in the system. It is assumed that the reaction conditions for each sample are the same throughout the decomposition.

Due to the Biotage® + Initiator reactor's capability to run a single sample under a highly controlled temperature and pressure this reactor is preferable for development of a procedure for the reaction of interest. In contrast, the MARS 5 system's batch approach is not preferential for development of procedure due to lack of monitoring of each individual sample. However, the MARS 5 system is beneficial in expanding methodologies due to the capability to run multiple samples per digestion.

To summarize, microwave instrumentation can vary significantly from one brand to another and may require specialized microwave vessels that differ in volume, shape, and ability to hold pressure. Overall, the high price tag for a microwave reactor, the availability of seemingly different types of microwave reactors, and lack of clarity of their relative comparison all can have limiting impact on a researcher's ability to transfer conventional heating method to a microwave reactor. Therefore, we investigated the benefits of microwave synthesis using Environmental Assessment Tool for Organic Syntheses (EATOS) calculations and compared the environmental impact of microwave synthesis and conventional experiments. EATOS software and the algorithm developed by Andraos. allows for the analysis of two metrics of synthesis: quantities of chemicals used, S^{-1} parameter, and waste produced, E factor, in a reaction [9].

EXPERIMENTAL DESIGN

Microwave experiments were performed using two most common microwave setups: a single-mode Biotage® Initiator+ synthesizer and a multi-mode MARS 5 digestion system. The Biotage® Initiator+ is equipped with a Robot Eight attachment allowing setup of multiple samples in a queue. Each sample was run individually using standard 3–5 mL single-use microwave vials recommended by manufacturer. The volumes of reactions were maintained between 2.5 to 3.5 mL to comply with safety requirements of the instrument. All microwave vials were capped prior to placing in the instrument's cavity. Reaction parameters were monitored using instrument interface program to ensure that the pressure did not exceed the safe limits and the temperature remained constant throughout the experiment. All syntheses involving MARS 5 digestion system were performed using 20 mL standard CEM vessels in a 16-vessel carousel. To ensure safe operation of the system the total volume across all samples were maintained at 20–30 mL according to the manufacturer guidelines. Individual reactions volumes kept at 5.0 to 7.0

mL to allow for the clear translation of reaction procedures between two reactors. All vessels were capped, however, due to the MARS vials' limitations the seal of the vials was not achieved. Several reactions were run side-by-side in the MARS reactor, the temperature probe was placed in the one to maintain temperature during the synthesis. No comparison between actual temperatures of other samples in the instrument cavity was made.

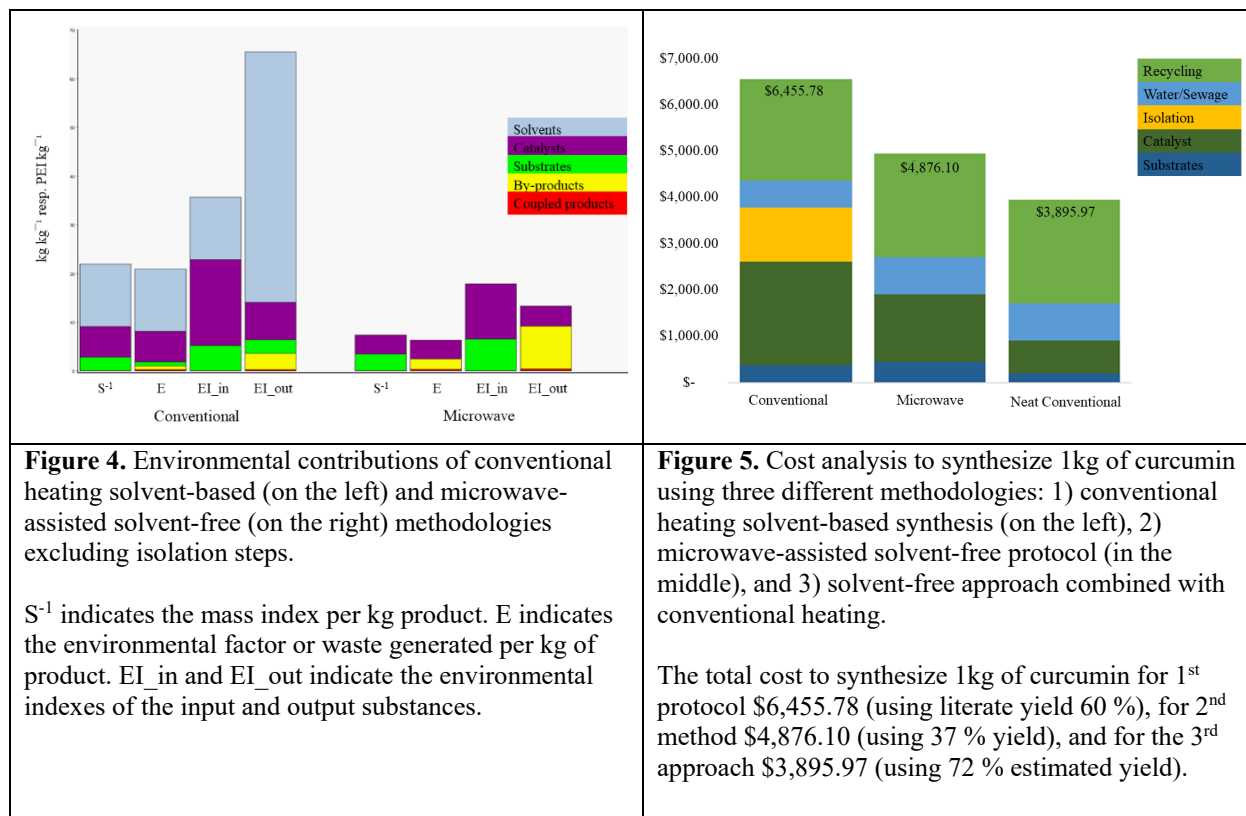
Typical synthesis using single-mode Biotage® Initiator+. The reactions were carried out using the solvent-free procedure developed previously in our lab for solvent-free curcuminoid synthesis using conventional heating [3]. The modifications were made to the isolation procedure to improve yields. Boric anhydride (0.1250 g, 1.80 mmol) and acetylacetone (0.257 mL, 2.50 mmol) were added into the microwave vial and stirred for 5 minutes at 350 rpm. After stirring an aldehyde (5.10 mmol), tri(n-butyl) borate (2.700, 10.0 mmol) and butylamine (0.100 mL, 1.00 mmol) were added to the microwave vial prior to sealing the vial. The vial was then placed on a stir plate and stirred for 15 seconds at 1200 rpm. The microwave vial was placed into the Robot 8 and the reaction was run for 85°C, 11:15 minutes, under high absorption, and 900 rpm stir rate, with activated cooling and FHT. Following the initial reaction and confirmation of product formation an ethanol digestion was run with the following conditions: 85°C, 22:32 minutes, high absorption, 900 rpm stir rate, cooling, and FHT. Product confirmation was completed using ¹H NMR (CDCl₃), TLC (hexane-acetone 2:1 eluent), and melting point. All signals in ¹H NMR spectra, melting point and R_f values for isolated compounds aligned with those previously reported [3].

Typical synthesis using multi-mode MARS 5 Digestion System. The reactions were carried using the two-fold increase of the quantities used for the single-mode microwave experiments. The order of addition of all reagents and the external parameters, e.g. temperature and time, were kept identical between two instruments. No comparison between the pressure values or actual samples' temperatures between two instruments were possible. The modifications to isolation procedure were kept to a minimum to ensure validity of the yield. Rotation of vials used for the monitoring of temperature was made occasionally for different trials, however, no systematic modification of a temperature probe vessel was attempted. Following the initial reaction and confirmation of product formation an ethyl acetate digestion was run with the following conditions: 85°C and 20 minutes. Product confirmation was completed using ¹H NMR (CDCl₃), TLC (hexane-acetone 2:1 eluent), and melting point. All signals in ¹H NMR spectra, melting point and R_f values for isolated compounds aligned with those previously reported [3].

¹H NMR monitoring experiments. Using solvent-free methodology to our advantage we conducted parallel monitoring of reaction progress using ¹H NMR spectroscopy for experiments carried out using Biotage® Initiator+ synthesizer. To analyze reaction mixture the reaction was prepared as usual in the microwave vial. After the selected time interval an 0.10 mL aliquot of reaction mixture was taken using syringe through septum on the vial cap and placed in the NMR tube along with CDCl₃ to a total volume of 0.60 mL. After locking and shimming spectra were collected at room temperature. Samples were not returned in the microwave vial upon data collection completion. Quantitative integration of C(O)-H signal of an aldehyde and the trans-coupling signals of corresponding product were used to collect data to estimate necessary reaction times (for values see above). After no change between aldehyde to product ratios was detected on NMR scale the reactions were quenched with water – ethanol mixture and product isolation was undertaken.

RESULTS

In figure 4 we demonstrate summarized results obtained using EATOS software for conventional and microwave synthesis of curcumin with reagent prices acquired from Aldrich on May 20, 2021 according to the manufacturer site. In figure 5 we use same software to analyze the cost of production for conventional method using ethyl acetate as a solvent and conventional heating, while the microwave method used solvent-free conditions and microwave heating. For the ease of comparison, we did not include voltage and power usage of each microwave reactor into the cost analysis. All of the cost comparison is based on our solvent-free method using Biotage single-mode reactor to allow one reaction to one reaction comparison.



In table 1 we summarize our findings on comparison of two instruments using vanillin, p-anisaldehyde and p-chlorobenzaldehyde as starting materials. The use vanillin provides curcumin as a final product, while other two aldehydes result in curcuminoids. We chose p-anisaldehyde to include a liquid starting material, and p-chlorobenzaldehyde as a solid starting material to allow analysis of how physical state impacts solvent-free method.

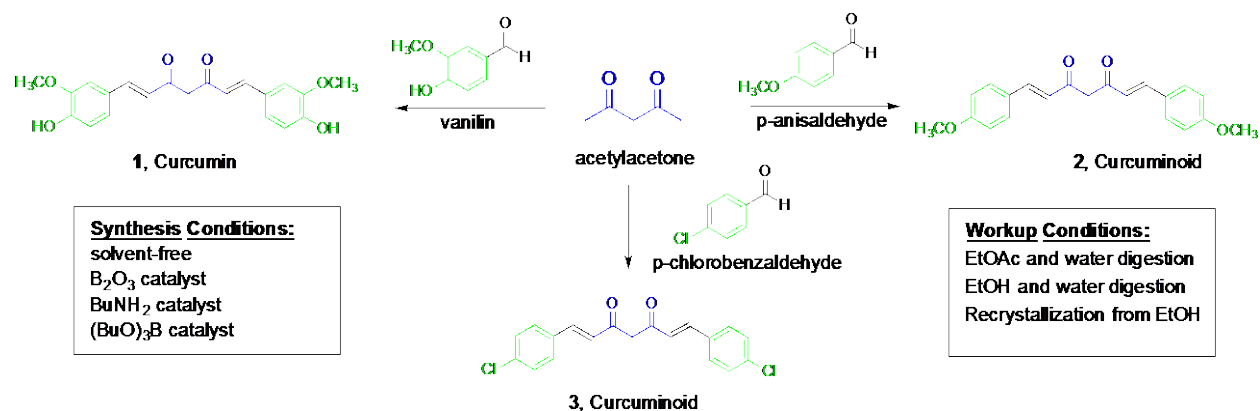
Table 1. Comparison of product yield.

Starting Aldehyde Used	Isolated Product Yield, %	
	Biotage® Initiator+ EtOH workup*	MARS 5 EtOAc/H ₂ O workup
p-anisaldehyde	47.3	82.1
p-chlorobenzaldehyde	24.7	75.2
vanillin	37.2	72.3

*Based on spectroscopic analysis of remaining filtrates and quantitative integration of C(O)-H signal of an aldehyde and the trans-coupling signals of corresponding product we estimate the actual yield to be as high by 30–40 %.

DISCUSSION

In this study we used synthesis of curcumin and two of its close analogs as modeled reactions (Scheme1). The reaction is carried out by combining a corresponding aldehyde (vanillin, para-anisaldehyde, or para-chlorobenzaldehyde) with a central linker, acetylacetone, in presence of several catalysts. The reasoning behind our choice of aldehydes is based on their physical state as solvent-free reactions have limited amount of liquid media present. After completion of reaction was confirmed using ¹H nuclear magnetic resonance spectroscopy (NMR) and thin-layer chromatography (TLC) the isolation of product was carried out using ethyl acetate and water or ethanol and water digestion. In some cases, recrystallization from ethanol was carried out to obtain highest purity samples.



Scheme 1. Summary of studied reactions, featuring synthesis of curcumin and its close analogs.

For environmental study we utilized all reagents listed on Scheme 1 for synthesis of curcumin **1**. When comparing the solvent-based synthesis with conventional heating to solvent-free microwave-assisted synthesis EATOS results in an approximate two-fold decrease in material necessary to make 1 kg product s^{-1} , waste per kg product E, and environmental index of input materials when using the microwave solvent-free synthesis (Figure 4). The largest change was with the environmental index of the output material which the microwave solvent-free method decreased by an approximate factor of 3.5 (Figure 4). EATOS was also used to calculate the cost to produce 1 kg of product from each method. The cost production of the solvent-based protocol with conventional heating decreased from \$6,455.78 to \$4,876.10 when using the microwave-assisted solvent-free synthesis (Figure 5). However, the solvent-free protocol with conventional heating provided the most cost-efficient method with a cost to produce 1 kg of product as \$3,895.97 (Figure 5). Results indicate that traditional solvent-based approach with conventional heating is both most harmful to the environment and less cost efficient.

The relative comparison of both instruments demonstrates their applicability for synthesis of curcumin and curcumin analogs. We found that use of Biotage® Initiator+ was very beneficial for establishing of the procedure, however, as expected, the use of MARS 5 allowed for quick acquisition of multiple data points. The isolated yields comparison, however, may be affected by the purification procedure (Scheme 1, Table 1) due to difference in reaction volumes and solubility of products in corresponding solvents used. For experiments conducted using MARS 5 the extraction of the product was conducted using ethyl acetate/water system, while ethanol digestion was used for Biotage® Initiator+. Although seemingly a minor substitution, this could serve as an explanation for generally lower isolated yields observed for single-mode microwave-assisted experiments, as reactions volumes were lower for these experiments and due to moderate solubility product remains in filtrate. The NMR monitoring data and quantitative integration of C(O)-H signal of an aldehyde and the trans-coupling signals of corresponding product we estimate the actual product yield are between 30 and 40% higher than isolated reported. Further investigation will need to be conducted to ensure that effect of different purification is factored in the comparison of two reactors.

LIMITATIONS

The following study was intended to test relative performance of the two reactors to identify best instrument to use for further method development using curcumin(oid) synthesis as model reactions. However, the sample size of target compounds in the study was left to be intentionally small to achieve fast comparison. This should be expanded to include other aldehydes to achieve a better understanding of patterns of yields. Purification methods used to isolate product were varied between two instruments. Future study should account for the purification solvent used. EATOS analysis was limited to curcumin itself and should be expanded to include analogs, e.g. curcuminoid **2** and **3** (Scheme 1), to provide a complete picture.

CONCLUSIONS

EATOS results indicated that microwave-assisted solvent-free synthesis is both economically and environmentally favorable to the traditional solvent-based protocol with conventional heating. Removal of solvent from synthesis of curcumin had the largest effect on the environmental impact of the methods as well as resulted in a substantial reduction of production cost. The comparison of single-mode Biotage® Initiator+ and batch mode MARS 5 microwave reactors demonstrated that both systems performed on similar level for synthesis of curcumin itself and two close analogs. Due to the differences acquired on isolation and purification steps the direct comparison of yields is not as informative and future study will be required to account for those factors.

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