Extra virgin olive oil jam enriched with cocoa bean husk extract rich in theobromine and phenols.

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**ABSTRACT**

Cocoa bean husk extract (CBHE) was obtained by thermal treatment at 170 °C for 30 minutes. The CBHE was added to a virgin olive oil jam in a freeze-dried form or encapsulated. Phenols, theobromine, and antioxidant activity were determined over 28 days in both jams and compared to a control jam. Total phenols, epicatechin, and theobromine were higher in the water fraction of the jam with non-encapsulated extract (J-CBHE) at day 28: 1140, 19.14 and 70 ppm, respectively. For the oil fraction of J-CBHE, total phenols, epicatechin, and theobromine contents were 120, 2.54, and 0.13 ppm at day 28, respectively. The oil content in jam with the non-encapsulated extract showed the best Rancimat induction time, with a 32% increase compared to the control, keeping for longer without becoming rancid. Nevertheless, the jam with encapsulated extract showed the best retention of bioactive compounds during the storage time.

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**Keywords:** Cocoa antioxidants, catechin, epicatechin, extra virgin oil, oxidative stability.
1. INTRODUCTION

The important role of food in supplying humans with more than just chemical energy is continuously being confirmed. An appropriate diet helps to reduce the risk of chronic lifestyle-related diseases such as cancer, diabetes, heart disease, atherosclerosis, hypertension, osteoporosis, and arthritis, among others (Arai, 2002). Reconsidering foods and their key components as the first line of defense against many diseases has become more common due to an increased understanding of their health benefits, resulting in the new concept of functional foods (Arai, 2005). In these context, a functional food is those edible product than besides to supplier chemical energy at the human body, it helps to reduce the risk of chronic life style-related diseases. Through the formulation of nutritionally improved products, functional foods may provide additional health benefits beyond the nutrients they traditionally contain (Jalili, Wildman, & Medeiros, 2001). In this sense, there is growing interest in studying new formulations to improve not only the stability of foods but also their functional properties. To this end, the use of phytochemical compounds with bioactive properties in food products may help to prevent the oxidation and spoilage of food, as well as improve nutritional and health benefits. Nowadays, many natural antioxidants are obtained from agroindustrial waste for application in the food industry, such as antioxidant extracts from olive oil waste (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2013) or grape waste (Angelov, Boyadzhiev, & Georgieva, 2016). One of the main aspects to bear in mind for food formulation is the oxidation of each component, with the most common heterogeneous matrix being lipid and aqueous fractions. With regards to lipid oxidation in foods, it is important to note that most kinds of oil-based foods are not found in their bulk form but are dispersed as colloidal particles in products such as dairy foods, processed meats, dressings, dips, beverages, desserts, yogurts and sauces. When lipids are dispersed as small particles in aqueous-based food products, there is a huge increase in the lipid-water surface
area. Reducing the dimensions of lipid particles in foods, as can occur in an oil-based jam, may also promote lipid oxidation because reactants such as hydroperoxides are surface active molecules that tend to accumulate at oil-water interfaces (Decker et al., 2017). In this context, Porter (1980) stated that polar antioxidants work best in bulk oils while non-polar antioxidants work best in lipid dispersions such as oil-in-water emulsions and liposomes. There are some explanations, one of which could be that the aqueous phase antioxidants are still able to scavenge free radicals at the interface, assuming that the fatty acid radicals are surface active; another possibility is that aqueous phase antioxidants could regenerate oxidized antioxidants at the lipid droplet surfaces (McClements, Decker, & Elias, 2010). In any case, it is crucial to find new sources of bioactive compounds for inclusion by the food industry as natural antioxidants in order to diminish the oxidation and increase the nutritional value of food formulations.

Cocoa bean husk (CBH) has long been considered an agroindustrial waste; nevertheless, several recent studies have addressed how to add value to this residue, highlighting its biological properties and potential health benefits (Okiyama, Navarro, & Rodrigues, 2017). The composition of CBH reported depends on the cocoa bean variety, country of origin, fermentation process and extraction methods employed (Wollgast, & Anklam, 2016; Oracz, Zyzelewicz, & Nebesny, 2015). In terms of bioactive compounds, it has been recently reported 17.70 ± 0.10 mg/g of epicatechin, 1.2 ± 0.1 mg/g of catechin and 12.00 ± 0.10 mg/g of the methylxanthine theobromine in cocoa bean husk of a Mexican fermented sample (Hernández-Hernández, Viera-Alcaide, Morales-Sillero, Fernández-Bolaños, & Rodríguez-Gutiérrez, 2018a). Later, Hernández-Hernández and colleagues obtained a CBH active extract using a simple and inexpensive industrial method (Hernández-Hernández et al., 2018b). They found a high correlation between total phenolic content and antioxidant activity, as measured by the DPPH method, in fermented CBH samples. Furthermore, (Hernández-
Hernández et al., 2018b) found that cocoa fiber obtained from cocoa husk might contribute to a reduction of cardiovascular risk as a result of its high content of dietary fiber and the presence of antioxidant polyphenols that may prevent lipid peroxidation (Lecumberri et al., 2007).

The aim of the present study was to utilize natural antioxidants present in a CBH extract to improve the heterogeneous food matrix formed by extra virgin olive oil (EVOO) and water. The use of a CBH extract for new food formulation was evaluated, including the optimal concentration of each main compound, the stability of bioactive compounds in the oil and aqueous phases, and the sensory properties of the jam. This heterogeneous food was chosen as one of the most representative kinds of foods in which the prevention of the oxidation of both phases, the storage life, and its nutritional properties are crucial. EVOO is rich in antioxidants that protect against lipid peroxidation, but the heterogeneous nature of the food matrix makes the EVOO more susceptible to oxidation. The use of an antioxidant-rich CBH extract to protect the EVOO in jam formulation (oil:water) is a good model to determine the effect of antioxidant addition in both the oil and aqueous phases.

2. MATERIALS AND METHODS

2.1. Plant material.

Theobroma cacao L. bean husk (Figure 1) (obtained from fermented, dried and pre-roasted beans) was provided by the cocoa factory Industria de Cacao de Tabasco Soc. Anónima (INCATABSA) located in Tabasco, Mexico. Extra virgin picual olive oil (EVOO) was provided by Centro Tecnológico del Olivar y el Aceite (CITOLIVA) located in Mengíbar, Jaén, Spain. Panela cane sugar Bio alternative ® was purchased from the local supermarket.
2.2. Chemicals.

The standards of gallic acid (PubChem CID: 370), (-)-epicatechin (PubChem CID: 72276), (+)-catechin hydrate (PubChem CID: 107957), theobromine (PubChem CID: 5429), trifluoroacetic acid (PubChem CID: 6422), Folin-Ciocalteu's phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (PubChem CID: 74358), maltodextrin and xanthan gum from *Xanthomonas campestris* were purchased from Sigma-Aldrich (Madrid, Spain). Na$_2$CO$_3$ (PubChem CID: 10340), methanol (PubChem CID: 887) were purchased from Panreac Química S.A. (Barcelona, Spain). Acetonitrile HPLC grade purity (PubChem CID: 6342) was purchased from Romyl, Teknokroma (Barcelona, Spain).

2.3. Cocoa bean husk extract (CBHE).

Cocoa bean husk (CBH) was ground by a LADY-MAX cooking mill. The enriched bioactive extract was obtained by mixing CBH and water in a 1:2 ratio, then the mix was added to a reactor at 170 °C for 30 minutes. Reactor characteristics have been previously described (Rubio-Senent, et al., 2013; Hernández-Hernández, et al., 2018b). The mixture was centrifuged at 4700 g (Comteifa, S.L., Barcelona, Spain) and, 2 L of the liquid phase was vacuum-filtered at room temperature using a kitasato flask and Büchner funnel. 0.5 L of the filtered elute was placed in a round flask, freeze-dried (Figure 1) by a lyophilizer Telstar LyoQuest (Spain). To encapsulate the extract with maltodextrin (CBHE+M), 10 g of maltodextrin was added to the concentrated extract before lyophilization.

2.4. Jam formulation.

Jams were made with the EVOO, panela cane sugar, xanthan gum and water. The ingredients were mixed with a thermomix ® TM31 electric mixer for 4 minutes at speed 5. Treatments and formulations in grams are outlined in Table 1.

2.5. Jam stability study.
The jam was stored in a closed bottles of polyethylene terephthalate (PET) without vacuum at 4 °C for 28 days. During this period, 10 g samples of each treatment were taken each 7 days (0, 7, 14 21 and 28 days) and stored at -20 °C until their analysis of total phenols, epicatechin and theobromine content, and antioxidant activity. 5 g of jam were centrifuged and the supernatant (oil fraction) was separated from the sediment (water fraction). 5 mL methanol:water 80:20 was added to the oil fraction with stirring for 30 minutes in a multi-rotator (Grant-Bio PTR-35, England). The extract was concentrated with a sample concentrator (block heater SBH130D/3, Stuart, UK) for 2 hours (at room temperature and nitrogen current). 2 mL methanol:water 80:20 was added and the extract centrifuged at 836 g for 10 minutes. The supernatant was recovered and stored at 4 °C until analysis. The procedure for extraction of the water fraction was similar but using concentrated methanol, then the supernatant was separated by the aid of fiberglass (SiO₂).

2.6. Concentration of CBHE in jams.

For the jam formulation, different tasting sessions were carried out by a panel of consumers with two proportions of the CBH additives: 0.25% (J-CBHE-a or J-CBHE+M-a) and 0.38% (J-CBHE-b or J-CBHE+M-b). Because of the similarity between the sensorial parameter of the olive oil jam and the olive oil, and because the main parameter was to evaluate the concentration of CBHE and not the final product, the test was carried out in a olive oil panel with six accredited panelist between 28 and 40 years old. The sensory attributes tested were odour, colour, flavour, texture, consistency, sweetness, bitterness and acceptability, which were evaluated with a 9-point Hedonic scale, where 1 refers to “I dislike it extremely” and 9 refers to “I like it extremely” similar than the used in the olive oil panel (Fernandes, Ellis, Gámbaro, & Barrera-Arellano, 2018). Further consumer tests will be necessary to assess the formulation of the commercial product.
2.7. Total phenols.

Total phenols were determined using the Folin-Ciocalteu's method. Samples of 20 µL were reacted with Folin-Ciocalteu’s reagent (Singleton & Rossi, 1965), and expressed as mg of gallic acid equivalents per gram. The absorbance was measured at 655 nm in a BIO-RAD iMark Microplate Reader (USA).

2.8. Antiradical activity by DPPH method.

Antiradical activity was determined reacting 5 µL of sample with 195 µL of a stock solution of 2, 2-diphenyl-1-picrylhydrazil as described in previous work (Hernández-Hernández et al., 2018b). Absorbance at 490 nm was measured after 30 min reaction time. The activity of each extract was expressed as an EC50 (effective concentration at 50% in mg/mL).

2.9. HPLC analysis.

The bioactive profile of both the water and oil fractions was performed using a Hewlett Packard Series 1100 liquid chromatography system equipped with a C-18 column (Kinetex® Biphenyl 100 Å, 250 mm× 4.6 mm, i.d. 5 µm), diode array detector and a rheodyne injection valve (20 µL-loop). The samples extracted from both fractions of the jam were filtered (0.45 µm) before injection into the HPLC. All compounds were detected at 280 nm as described by Hernández-Hernández et al. (2018a). The mobile phases were 0.01 % trichloroacetic acid in water (B) and acetonitrile (A), using the following gradient over a total run time of 55 min: initially 5% A, 25% A, 50% A, 100% A, 25% A, 5% A and 95% B, 75% B, 50% B, 0% B, 75% B, 95% B. Calibration curves were constructed for theobromine and (-)-epicatechin at concentrations ranging from 0 to 2 mg/mL ($r^2 \geq 0.99$) and from 0 to 1 mg/mL ($r^2=0.99$) respectively. All determinations were carried out in duplicate.
2.10. **Determination of oxidative stability in the oil fraction of the jam by the Rancimat Method.**

Lipid oxidative stability of the EVOO fraction of each jam was evaluated by an accelerated automated test using the Rancimat equipment (Model 679, Metrohm Co. Basel, Switzerland) as previously described (Mrabet et al., 2017). Aliquots of 2.5 g of each jam’s oil fraction were placed in the equipment. The temperature and the air flow were adjusted for each sample to 120 °C and 10 mL/min of air. The induction times were printed automatically by apparatus software with an accuracy of 0.005. Results were expressed as induction time (IT) in hours and the percentage in which this time was improved by the extracts. The protective index (PI) was calculated by dividing the antioxidant-enriched oil fraction’s IT value by the control’s IT value. All determinations were carried out in duplicate.

2.11. **Statistical analysis.**

Results were expressed as mean values ± standard deviations. STATGRAPHICS® plus software was used for statistical analyses. Comparisons amongst samples were made using one-way analysis of variance (ANOVA) with Student’s t test and LSD method at the same confidence level. A p value of <0.05 indicated a statistically significant differences.

3. **RESULTS AND DISCUSSION**

3.1. **Concentration of CBHE in jams.**

The two types of extract CBHE and CBHE+M were added at 0.25% and 0.38% to extra virgin olive oil jam. The sensory attributes were scored by a panel of consumers (Figure 1). There were clear differences between the sensory attributes of the jams,
which were particularly evident in the flavour, texture, sweetness and bitterness. Colour, texture and consistency were evaluated with the lowest score in the J-C (control), which means the panel rates these attributes more highly for the jams with cocoa bean husk extracts added, with and without maltodextrin. Jam with 0.25% CBHE (J-CBHE-a and J-CBHE+M-a) obtained the best scores for odour, colour and flavour. These results are in agreement with those reported by Spinelli, Lecce, Likyova, Del Nobile & Conte (2018), who studied the addition of different percentages of microencapsulated orange extract to a fish burger finding that its sensory properties, including odour, colour, texture and overall quality, were affected.

3.2. Stability of jams.

Due to the positive sensory analysis, the jam with 0.25% of extract added was selected to be evaluated over 28 days of storage for the stability of total phenols, epicatechin, and theobromine. The antioxidant capacity and the oxidative stability were also determined by DPPH and Rancimat methods, respectively. The concentration of total phenols, bioactive profile (theobromine and epicatechin) and the anti-radical activity were evaluated in both the water and oil fraction every 7 days. The concentration/content of total phenols (Figure 2) increased with the addition of freeze-dried CBHE (J-CBHE) in both fractions, but no significant differences were observed when the extract added was capsulated in maltodextrin (J-CBHE+M) with respect to the control jam (J-C). In the water fraction of the J-CBHE, a higher content of total phenols was recorded during the entire evaluation period, although the phenol content dropped during the storage period from about 1700 to 1100 ppm (Figure 2a). In contrast, the total phenol content in the water fraction of J-C and J-CBHE+M remained very similar during the 28 days (decreasing from 800 to 600 ppm for J-CBHE+M). In the oil fraction of J-CBHE a remarkable and curious increase in the
phenolic content was observed during the second half of the storage time, peaking at 21 days, although it decreased at the final stage (Figure 2b). Total phenols in the oil fraction were detected below 50 ppm throughout the entire period for both J-C and J-CBHE + M. The concentration of total phenols was much higher than for epicatechin during the storage period (Figure 3), despite the fact that epicatechin is the most abundant phenol in CBH. This could be due to the presence of polymerized phenols or phenolic compounds linked to sugars and/or proteins in both the extract and the final jam.

The concentrations of epicatechin (Figure 3a) and theobromine (Figure 4a) diminished from 0 to 28 days of storage in the water fraction. J-CBHE consistently showed the highest contents; however, greater reductions for J-CBHE than J-CBHE+M were observed during the storage period. This behaviour could be due to the advantage conferred by the maltodextrin encapsulation in the conservation of bioactive compounds, as found for the encapsulation of green tea extract (Silva et al., 2018). In the oil fraction, although the contents of epicatechin (Figure 3b) and theobromine (Figure 4b) were lower than those found in the water fraction, less than 2.5 ppm and 0.18 ppm, respectively, the contents of both compounds were considerably higher in J-CBHE. It is important to note that although theobromine was found in higher concentrations than epicatechin in the water fraction of both treatments (J-CBHE and J-CBHE+M), theobromine was present in lower concentrations in the oil fraction of both treatments than epicatechin. This could be due to the fact that epicatechin has a higher oil solubility than theobromine (Aditya et al., 2015; Spiller 1997).

The same amount of freeze-dried extract (J-CBHE) was added as of extract encapsulated in maltodextrin (J-CBHE+M), thus the amount of bioactive compounds present in the J-CBHE+M extract was lower than for J-CBHE. Despite this, two clearly different behaviors were observed in the oil and in the aqueous fractions. In the oil fraction, the addition of the non-encapsulated extract (CBHE) increased the content of
total phenols and of bioactive compounds analyzed, theobromine and epicatechin, much more so than to the addition of encapsulated extract (CBHE+M) (Figures 2b, 3b and 4b). This indicates that these components are transferred to the oil phase and that their encapsulation makes the transfer more difficult. This hypothesis was confirmed by the Rancimat test, in which the induction time of oxidative stability was greater in the oil fractions of extract added without encapsulation (12.85 h) compared to the oil fraction with encapsulated extract (8.44 h), a 32.61% increase in the induction time compared to the control (Figure 5).

Similarly, the quantities of total phenols, theobromine, and epicatechin in the aqueous phase of the non-encapsulated extract were higher than in the encapsulated ones; however, the levels diminished during the analyzed time (Figures 2a, 3a and 4a). Thus it seems that encapsulation favours the stabilization of these components in the aqueous phase, a result that is supported by the antioxidant activity test (Figure 6), where the highest value was found after 28 days for the jam with encapsulated extract. No other major differences were found between the samples for all jams (Figure 6).

Several studies have affirmed the protective effects of different encapsulation techniques, such as extrusion and spray drying, from food destructive factors including moisture and heat (Arshady, 1993; Chang & Nickerson, 2018; Chen et al., 2018; Lupo, Maestro, Gutiérrez & González 2015). In agreement with two studies (Chang & Nickerson, 2018; Da Silva et al., 2014) the main factors affecting the preservative and release characteristics of encapsulated extracts are related to interactions between wall and core materials, physical properties of microcapsules, viscosity and solubility of wall materials, and the structure of microcapsules.

From these results we suggest that cocoa bean husk additive improves the useful life of the jam, a heterogeneous mixture of oil and aqueous material. Also, the use of the extract without encapsulation is more suitable for the formulation of food with a higher
fat content since it favours solubilization in the oil phase, while for foods with a higher proportion in water, encapsulation improves the water stability of the added bioactive compounds. Future studies should focus on the addition of the two types of extracts, to investigate the synergy of both types in one food matrix.

It is important to point out that despite the use of an extra virgin olive oil-based jam, itself rich in phenols, the positive effect on oxidative stability resulting from the addition of cocoa extract was clear. Therefore, its use in other types of fat- and oil-based foods whose antioxidant contents are lower would be even more significant. For instance, other authors have reported the use of phenols such as catechin to prevent the depletion of the oxidative activity of cotton seed oil (Tsimogiannis & Oreopoulo, 2007). Also, other authors (Belhaj Arab-Tehrany, & Linder, 2010) proved that the addition of quercetin improved the oxidative stability of bulk salmon oil, although the same effect was not observed in emulsion due the insolubility of this compound in water and lipid phases.

4. CONCLUSIONS

The use of cocoa bean extract improved the stability and antioxidant activity of the formulated food, through the solubilization of total phenols, theobromine, and epicatechin. For foods with a higher content of fat or oil, it would be more appropriate to add the cocoa extract without encapsulation, and in the case of foods richer in the aqueous phase, the addition of an encapsulated extract would be more effective. Further tests are necessary to determine if the addition of encapsulated or non-encapsulated cocoa bean husk extract could improve a heterogeneous food depending on the water: oil ratio.
Thus, the addition of bioactive extracts obtained from industrially-treated cocoa bean husk can enhance the stabilization of the oil and aqueous phases of heterogeneous foods.

ACKNOWLEDGEMENTS

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2. REFERENCES


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Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International, 33*(6), 423–427. [https://doi.org/10.1016/S0963-9969(00)00068-5](https://doi.org/10.1016/S0963-9969(00)00068-5).
**Figure captions**

**Figure 1.** Cocoa bean husks (A), the cocoa bean husk extract (B) and the sensory properties of jams enriched at two different concentrations: 0.25% (-a) or 0.38% (-b) with freeze-dried cocoa bean husk extract (J-CBHE) (a ■; b ■) or cocoa bean husk encapsulated extract (J-CBHE+M) (a ■; b ■). J-C: Control jam (no CBHE) (■).

**Figure 2.** The evolution of total phenols (ppm) determined by HPLC in the water (a) and oil (b) fractions of jams enriched with freeze-dried cocoa bean husk extract (J-CBHE) (- ■ -), cocoa bean husk encapsulated extract (J-CBHE+M) (..▲..) and the control without CBHE (J-C) (-→-). An asterisk indicates a significant difference between enriched jams (J-CBHE and J-CBHE+M) and the control (J-C) at P < 0.05 by Tukey’s test after analysis of variance.

**Figure 3.** The evolution of epicatechin (ppm) determined by HPLC in the water (a) and oil (b) fractions of jams enriched with freeze-dried cocoa bean husk extract (J-CBHE) (- ■ -) and cocoa bean husk encapsulated extract (J-CBHE+M) (..▲..). An asterisk indicates a significant difference between J-CBHE and J-CBHE+M at P < 0.05 by Tukey’s test after analysis of variance.

**Figure 4.** The evolution of theobromine (ppm) determined by HPLC in the water (a) and oil (b) fractions of jams enriched with freeze-dried cocoa bean husk extract (J-CBHE) (- ■ -) and cocoa bean husk encapsulated extract (J-CBHE+M) (..▲..). An asterisk indicates a significant difference between J-CBHE and J-CBHE+M at P < 0.05 by Tukey’s test after analysis of variance.
Figure 5. Induction time (IT) of the oxidative stability of the oil fraction of jams by Rancimat assays. PI: protective index.

Figure 6. Antiradical activity (DPPH assay) of the water fraction of jams, expressed as EC50 (mg/mL). Liquid fraction from the jams enriched with freeze-dried cocoa bean husk extract (J-CBHE) (- ■ -), cocoa bean husk encapsulated extract (J-CBHE+M) (-▲-) and the control without CBHE (J-C) (--♦--). An asterisk indicates a significant difference between enriched jams (J-CBHE and J-CBHE+M) and the control (J-C) at P < 0.05 by Tukey's test after analysis of variance.
**Table 1.** Treatment description and jam formulations (in grams) of jam control (J-C), jam with freeze-dried cocoa bean extract (J-CBHE) or jam with encapsulated cocoa bean extract (J-CBHE+M).

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Jam control (J-C)</th>
<th>Jam with CBHE (J-CBHE)</th>
<th>Jam with CBHE+M (J-CBHE+M)</th>
</tr>
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<tbody>
<tr>
<td>EVOO</td>
<td>50.00</td>
<td>50.00</td>
<td>50.00</td>
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<tr>
<td>Sugar</td>
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<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Water</td>
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<td>25.00</td>
<td>25.00</td>
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<tr>
<td>Xanthan gum</td>
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<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>CBHE</td>
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<td>0.25-0.38</td>
<td>0.00</td>
</tr>
<tr>
<td>CBHE+M</td>
<td>0.00</td>
<td>0.00</td>
<td>0.25-0.38</td>
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</table>
Figure 1.
Figure 2.
Figure 3.
Figure 4.
<table>
<thead>
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<th>Treatment</th>
<th>Induction time (h)</th>
<th>Δ IT (%)</th>
<th>PI</th>
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<tr>
<td>J-C (Lines 1 and 2)</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J-CBHE + M (Lines 3 and 4)</td>
<td>8.44 ± 0.23</td>
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<td>0.87</td>
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<tr>
<td>J-CBHE (Lines 5 and 6)</td>
<td>12.85 ± 0.21</td>
<td>32.61</td>
<td>1.33</td>
</tr>
</tbody>
</table>

*Figure 5.*
Figure 6.