
Optimization of phytocannabinoid extraction from hemp (*Cannabis sativa* L.) with decarboxylation-based crude palm kernel oil and its potential as an energy supplement product for suckling piglets

Kongkeaw, A., Charoensook, R., Incharoen, T., Hwanhlem, N. and Tartrakoon, W.*

Division of Animal Science and Feed Technology, Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand.

Kongkeaw, A., Charoensook, R., Incharoen, T., Hwanhlem, N. and Tartrakoon, W. (2025). Optimization of phytocannabinoid extraction from hemp (*Cannabis sativa* L.) with decarboxylation-based crude palm kernel oil and its potential as an energy supplement product for suckling piglets. *International Journal of Agricultural Technology* 21(3):903-926.

Abstract This study investigated the extraction of phytocannabinoids from *Cannabis sativa* L. (hemp) using medium-chain triglyceride (MCT) oil to evaluate their potential as energy supplements for suckling piglets and MCT emulsion formula with phytocannabinoids and highly concentrated lauric acid (EMPL prototype product). The most effective conditions were found at 12% hemp leaves in CPKO with a 6-hour heating period, resulting in the highest TPC (57.38 mg GAE/g DW), and antioxidant activities (DPPH: 34.43%, ABTS: 64.33%, and FRAP: 53.18%) ($P < 0.01$). The obtained extract contained 0.016% cannabidiol (CBD), 0.015% cannabidiolic acid (CBDA), 0.029% total cannabidiol, along with 54.50% medium-chain fatty acids, 47.09% lauric acid, 96.48% total fat, and a gross energy content of 8,813 kcal/kg. The EMPL group showed significantly higher performance in average weight (2.00 kg), average daily gain (ADG) (132.59 g/piglet/day), and milk intake (552.01 ml/piglet) at 5 days old compared to the control group ($P < 0.01$). These findings indicated that 12% hemp leaves extract in CPKO, heated for 6 hr at 110°C, significantly enhances antioxidant and phytocannabinoid content. An emulsion of medium-chain fatty acids with 40% lauric acid plus phytocannabinoids (EMPL) improved the growth performance and milk intake of piglets, demonstrating its potential as an effective and innovative energy supplement for suckling piglets.

Keywords: Antioxidant potential, Decarboxylation, Chemical composition, Fatty acid, Suckling piglets

Introduction

Cannabis sativa L. (hemp) has garnered considerable attention due to its various applications in agriculture, industry, medicine, herbal remedies, and

*Corresponding Author: Tartrakoon, W.; Email: wandeeta@nu.ac.th

religious practices. (Andre *et al.*, 2016) A critical aspect of hemp processing involves the extraction of phytocannabinoids, which is typically carried out through decarboxylation—a process using heating in combination with solvents such as cold-pressed coconut oil. Decarboxylation consists of three main stages: material preparation, cannabinoid activation through heating, and extraction (Moreno-Sanz *et al.*, 2020; Valizadehderakhshan *et al.*, 2021). The first step, material preparation, entails selecting high-quality cannabinoid-rich hemp. The chosen plant material is then dried and ground to increase surface area, which enhances extraction efficiency by reducing excess moisture that may impede the decarboxylation process. The second step is an activation process of cannabinoids by heating and controlling pressure conditions in autoclave sterilization. This process is the transformation of acidic forms like tetrahydrocannabinol acid (THCA) and Cannabidiolic acid (CBDA) into their active counterparts, Tetrahydrocannabinol (THC) and Cannabidiol (CBD), by removing a carboxyl (-COOH) group and CO₂ (Nachnani *et al.*, 2021; Fućak *et al.*, 2023; Nguyen *et al.*, 2024.). Typically, this step involves heating the material to temperatures between 105°C and 120°C for 30 to 60 minutes (Moreno *et al.*, 2020; Regan *et al.*, 2022). The final step is an extraction that uses carrier oil, such as crude palm kernel oil (CPKO), to dissolve the cannabinoids. The oil is heated to the appropriate temperature to facilitate the process, followed by filtration and then purification which concentrates or isolates specific cannabinoids for further use (Fućak *et al.*, 2023). There are several important factors to consider for the decarboxylation process, including the preparation of hemp material, precise control of heating temperature and duration to avoid overheating, and careful monitoring of equipment such as ovens and moisture control systems. The cooling down of the material after activation is also crucial. Furthermore, the post-decarboxylation steps must be tailored to each product's specific targets and manufacturing processes (Valizadehderakhshan *et al.*, 2021).

Hemp is a rich source of bioactive compounds, particularly phytocannabinoids, which have been extensively studied for their therapeutic potential. Several research studies focused on the product's components, including hemp derivatives. Cannabidiol (CBD) and tetrahydrocannabinol (THC) are becoming increasingly important because they have the potential to be treated or applied in both humans and animals. Recently, attention has shifted toward the role of non-psychoactive phytocannabinoids as functional ingredients in animal nutrition, particularly for enhancing livestock health and performance (Rocca and Salvo, 2020. According to a study by Lust *et al.* (2023), who explored the use of phytocannabinoids from hemp in combination with medium-chain fatty acids (MCFAs) by, formulating as triglyceride powders and monoglyceride emulsions (Bartoncíková *et al.*, 2023; Jadhav and Annapure, 2023), which aimed to use this

combination as a livestock additive for key nutritional needs in piglets (Jackman *et al.*, 2020). Four objectives have been reported for such a utilization model: enhancing growth efficiency, promoting weight gain, optimizing lipid absorption, and supporting digestive system development. These objectives target the improvement of energy availability and intestinal health while fostering microbial diversity and pathogenic control. Finally, these contributions will increase vitality and improve response behavior in piglets and pets (Vodolazska and Lauridsen, 2020; Rocca and Salvo, 2020; Montero *et al.*, 2023).

Therefore, this study aimed to evaluate the effectiveness of hemp material and medium-chain triglyceride (MCT) oil used as solvents for phytocannabinoid decarboxylation and extraction. A prototype emulsion combination of phytocannabinoid extracts and concentrated lauric acid, a novel energy supplement for suckling piglets to serve as an oral administration, and its impact on the productive performance was also assessed.

Materials and methods

Experiment 1: Extraction of phytocannabinoids from hemp roots and leaves using different MCT oils

The experiment used a 2x2 factorial in Completely Randomized Design (CRD) consisted of factor A is raw materials from hemp (hemp leaves and roots), and factor B was the type of triglyceride oil with medium-chain fatty acids, purified palm kernel oil (PPKO) and CPKO.

The soil remaining on the hemp roots was washed with tap water. The washed roots and leaves were then dried using a solar dryer. The raw materials were carefully selected and ground using an herbal grinder. The hemp fibers were separated manually. The grounded sample was sifted through a 100-mesh (150-micron). Subsequently, the cannabinoid content (%w/w) of hemp leaves and roots, including cannabidiol (CBDV), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabinol (CBN), tetrahydrocannabivarin (THCV), tetrahydrocannabinol (THC), cannabichromene (CBC), and tetrahydrocannabinolic acid (THCA), was determined through spectral analysis using High-Performance liquid chromatography (HPLC), based on a modification of AOAC Official Method 2018.11, Revised First Action by Vaclavik *et al.* (2019). The chemical composition, including ash, energy, carbohydrates, crude fiber, fat, protein, and moisture, was estimated using standard methods performed by Central Laboratory (Thailand) Co., Ltd., Chiang Mai Branch, as presented in Table 1.

Twelve grams of finely ground hemp leaves and roots were transferred into cloth bags (5 × 15 cm) at a 6% (w/v) ratio in oil solvent. Twelve bags (for four treatment comparisons, with 3 replications) were wrapped in aluminum foil and

subjected to sterilization in an autoclave at 121°C for 21 minutes, 0.11 MPa, which not only ensured the sterility but also promoted the decarboxylation of the raw material. Glass containers (250 ml) containing oil solvents (PPKO and CPKO) were also sterilized under the same conditions. After sterilization, the sterilized cloth bags were placed into the containers, and 200 ml of oil solvent was added. Emulsifiers (Tween 80, 2% w/v) and sodium erythorbate (0.3% w/v) were added to the solution and mixed to prevent rancidity (Tymoszczyk, 2013). The containers were sealed and stored in a dark place for 18-20 h to facilitate extraction, followed by heating at 110°C for 4 hours in a hot air oven. (Fućak *et al.*, 2023; Ryu *et al.*, 2021). After heating, the containers were cooled to 30 - 35°C in a dark room. The phytocannabinoid-enriched oil was then filtered through 100-mesh nylon, packed in opaque containers, and stored at room temperature (25±2°C). The antioxidant properties of the phytocannabinoid extracts were subsequently analyzed, including the antioxidant properties of the phytocannabinoid-rich oil by measuring total phenolic content (TPC) (mg GAE/g DW), total antioxidant capacity (TAC) in L-ascorbic acid (mg GAE/g DW), and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays (%) (Benkhaira *et al.*, 2022; Minarti *et al.*, 2024).

Experiment 2: Effect of duration of the decarboxylation and the extraction ratio of hemp to CPKO on the phytochemical composition and antioxidant potential of phytocannabinoid oil extracts

This study employed a 3×5 factorial arrangement in CRD to evaluate the effects of two key factors: (A) the ratio of hemp leaves to crude palm kernel oil (CPKO) and (B) the decarboxylation reaction time. Factor A consisted of three levels: A1 = 6%, A2 = 12%, and A3 = 18% (w/v in oil solvent), while Factor B included five levels of decarboxylation reaction time: B1 = 0 h (control), B2 = 2 h, B3 = 4 h, B4 = 6 h, and B5 = 8 h. The grounded hemp leaves prepared previously (experiment 1) were weighed at 12, 24, and 36 grams and transferred into cloth bags (5 × 15 cm), achieving concentrations of 6%, 12%, and 18% (w/v in oil solvent). This step obtained 45 bags (15 treatment comparisons with 3 replications were prepared). The badge was wrapped in aluminum foil and subjected to an autoclave at 121°C for 21 minutes to promote decarboxylation, sterilize the material, and accelerate the activation reaction. This procedure facilitated decarboxylation to ensure the sterility of the materials and accelerated the activation of the target compounds. They were autoclaved and placed into glass containers. To prevent oil rancidity, 4 ml of emulsifier (Tween 80,

equivalent to 2% w/v in oil solvent) and 0.6 g of sodium erythorbate (0.3% w/v in oil solvent) were added to the glass containers (Tymoszczyk, 2013). The containers were sealed tightly and stored in a dark environment for 18-20 h.

The extraction was conducted by heating at 110°C for 0, 2, 4, 6, and 8 h in a hot air oven (Fućak *et al.*, 2023; Ryu *et al.*, 2021). The extracted sample was cooled to 30 - 35°C in a dark place, The extracted oil was then filtered using a 100-mesh nylon filter and stored in opaque containers at room temperature (25±2 °C). The antioxidant properties of the phytocannabinoid-rich oil were evaluated by measuring TPC (mg GAE/g DW), TAC in L-ascorbic acid (mg GAE/g DW), and antioxidant activity using DPPH, ABTS, and FRAP assays (%) (Benkhaira *et al.*, 2022; Minarti *et al.*, 2024).

Experiment 3: Effects of oral administration of EMPL product prototype on body weight, growth rate, colostrum intake, and milk intake in suckling pigs

Animals and housing

Seventy newborn piglets were randomly selected. A 1.0 – 1.5 kg piglets born from sows with an average parity of 3 was divided into two groups. The control group (CON) received an oral dose of 2 ml of toltrazuril (50 mg/ml; commercial product toltrazuril 5%, registration number 1D 64/54), Better Pharma Company Limited, Thailand. The treatment group was orally administered 3.5 ml of EMPL. Both groups were treated twice: the first administration occurred 12–24 h after birth, and the second on the third day postpartum.

The piglet's weight was recorded immediately after birth, at 24 h. On the fifth day postpartum, the weight gain and average daily gain (ADG, g/day) 24h and 5-day, colostrum intake (ml/piglet), and milk intake on day 5 (ml/piglet) were evaluated and recorded. Sows and piglets were housed individually in farrowing pens (2 m × 2.2 m) equipped with heating lamps, rigid plastic walls, partly solid concrete flooring combined with hard plastic slats, and provided with straw bedding. Both water and straw bedding were supplied ad libitum, while the ambient temperature was maintained at 28±°C initially.

All procedures employed in this research were approved by the Naresuan University Animal Care and Use Committee (ACUC) under document number 67 01 002. The experiment was conducted at Wilaiporn Farm, Nakhon Sawan Province, Thailand, with research and testing certified by the Department of Livestock Development and under veterinary supervision.

Preparation of EMPL adjuvant prototype

This prototype was developed as an innovative energy supplement to enhance suckling piglets' nutritional profile and growth performance. A prototype

energy supplement for suckling piglets, EMPL (Emulsion of Medium-Chain Fatty Acids with 40% Lauric Acid plus Phytocannabinoids, Em-MCFA-Phyto-L40) was formulated. The emulsion contains 25 - 29% phytocannabinoid extract obtained from CPKO, and other ingredients include rice bran oil, palm oil, soybean oil, lauric acid powder (99.9% purity), polysorbate (Tween 80), butylated hydroxytoluene (BHT), benzoic acid, and sterile water.

Statistical analysis

The data from Experiment 1 was analyzed using a 2×2 factorial in CRD, while Experiment 2 employed a 3×5 factorial in CRD, which was subjected to analysis of variance (ANOVA). Group comparisons were performed using Tukey's Honestly Significant Difference (HSD) test, with statistical significance established at $P < 0.05$. A t-test was employed to compare the mean values between the control and EMPL groups in Experiment 3, with results presented at a 95% confidence interval. All statistical analyses were conducted using Jamovi software (version 2.3.28) (The Jamovi Project, 2022; R Core Team, 2021; Fox and Weisberg, 2020; Length, 2020).

Results

The phytochemical composition of hemp leaves and roots, after preparation and activation of the raw materials, revealed differences in the levels of CBD (0.168% in leaves and 0.073% in roots) and CBDA. (0.083% in leaves and 0.267% in roots), as shown in Figure 1 and Table 1. In terms of the chemical composition of both hemp materials, the levels of energy, carbohydrates, and protein were higher in leaves than in roots—297.25 kcal/100g vs. 107.79 kcal/100g, 49.56 g/100g vs. 23.04 g/100g, and 20.06 g/100g vs. 2.02 g/100g, respectively. In contrast, the crude fiber content was higher in roots than in leaves, at 45.34 g/100g and 12.80 g/100g, respectively (Table 1).

The extraction of hemp leaves and roots was investigated in conjunction with triglyceride oils rich in medium-chain fatty acids, namely PPKO and CPKO, to assess their antioxidant properties. A significant interaction ($P < 0.01$) between the type of hemp material and the medium-chain triglyceride oil used was observed. Extraction using hemp leaves with both PPKO and CPKO as solvents significantly yielded higher TPC and TAC than extractions using hemp roots ($P < 0.01$). Moreover, the combination of hemp leaves and CPKO exhibited the highest DPPH, ABTS, and FRAP antioxidant activity among all experiments ($P < 0.01$) as presented in Table 2.

Table 1. The phytochemical and nutritional composition of hemp leaves and roots

Items	Hemp materials	
	Leaves	Root
Cannabinoids (%w/w)^{1/}		
Cannabidiarin (CBDV)	0.014	0.001
Cannabidiolic acid (CBDA)	0.083	0.267
Cannabidiol (CBD)	0.168	0.073
Cannabinol (CBN)	0.007	0.005
Tetrahydrocannabivarin (THCV)	0.007	0.001
Tetrahydrocannabinol (THC)	0.017	0.048
Cannabichromene (CBC)	0.013	0.002
Tetrahydrocannabinolic acid (THCA)	0.005	0.018
Chemical composition^{2/}		
Ash ^{3/} (g/100g)	23.49	6.52
Energy ^{4/} (kcal/100g)	297.25	107.79
Carbohydrate ^{5/} (g/100g)	49.56	23.04
Crude Fiber ^{6/} (g/100g)	12.80	45.34
Fat ^{7/} (g/100g)	7.05	4.87
Protein ^{8/} (g/100g)	20.06	2.02
Moisture ^{9/} (g/100g)	11.01	6.74
Antioxidant efficiency		
Total Phenolic Content (TPC) ^{10/} (mg GAE/g DW)	70.81	33.82
Antioxidant potential capacity (APC) ^{11/} (mg GAE/g DW)	23.26	10.79
Antioxidant activity on DPPH reaction (ADA-DPPH)	28.56	26.86

^{1/} Cannabinoids: Cannabis Extraction and Analysis Laboratory (Modification of AOAC Official Method 2018.11, Revised First Action by Vaclavik *et al.* (2019)

^{2/} Central Laboratory (Thailand) Co., Ltd. ^{4/} Energy: In-house method TE-CH-169 based on method of Analysis for Nutrition Labelling (1993) pp.106, ^{5/} Carbohydrate using methods in Chapter 6: Proximate and mineral Analysis. (AOAC, 1993) ^{3/} Ash: AOAC Method 923.03 and 920.153, ^{6/} Crude Fiber: AOAC Method 978.10, ^{7/} Fat: AOAC Method 948.15, ^{8/} Protein: AOAC Method 991.20, ^{9/} Moisture: AOAC Method 925.10 and 950.46 according to AOAC. (2019) ^{10/} $y = 0.0008x + 0.0402$; $R^2 = 0.9911$ ^{11/} $y = 0.0014x - 0.003$; $R^2 = 0.9946$

In Experiment 1, hemp leaves were extracted using CPKO as the solvent, with the raw material to solvent ratio optimized to 6% (w/v). The activation of the raw materials was heated, pressured, and steam sterilized in an autoclave to induce decarboxylation before extraction. The extraction was conducted in sealed glass containers at a constant temperature of 110°C for 4 h, facilitating the efficient transfer of bioactive compounds from the leaves into the CPKO. To minimize contamination and reduce post-extraction filtration, hemp leaves were enclosed in cloth bags. The methodology employed, including using hemp leaves with CPKO and specific preparation techniques, provides a foundation for future research to

optimize the hemp leaves into solvent ratio and extraction duration. These parameters were further investigated in Experiment 2, as outlined in Table 2.

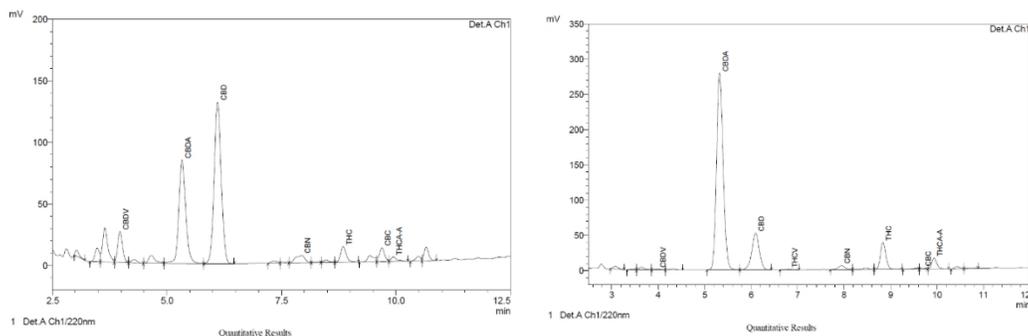
Table 2. Hemp extract combined with medium-chain fatty acid triglyceride oil on the antioxidant potential of phytocannabinoid oil extracts

Factor	TPC mg GAE/g DW	TAC mg GAE/g DW	DPPH assay (%)	ABTS assay (%)	FRAP Assay (%)
Interaction between factor A x B					
Hemp leaves x PPKO	46.15±0.04 ^a	24.33±0.76 ^a	17.29±0.25 ^c	44.52±0.50 ^b	36.77±0.42 ^b
Hemp leaves x CPKO	48.34±0.30 ^a	25.65±0.77 ^a	26.48±0.58 ^a	46.40±0.53 ^a	38.33±0.43 ^a
Hemp root x PPKO	30.56±0.45 ^b	9.57±0.51 ^b	14.58±0.61 ^d	28.17±0.06 ^c	19.60±0.55 ^d
Hemp root x CPKO	26.67±1.53 ^b	9.35±0.15 ^b	19.23±0.68 ^b	23.73±0.66 ^d	23.26±0.05 ^c
Factor A: Hemp raw materials					
Hemp leaves	47.25±1.22 ^a	24.99±0.99 ^a	21.89±5.05 ^a	45.46±1.13 ^a	37.55±0.93 ^a
Hemp root	28.61±2.36 ^b	9.46±0.36 ^b	16.90±2.61 ^b	25.95±2.46 ^b	21.43±0.23 ^b
Factor B: Types of triglyceride oils containing medium-chain fatty acids					
Purified palm kernel oil (PPKO)	38.36±8.54	16.95±8.11	15.94±1.54 ^b	34.13±11.39 ^b	28.189±9.41 ^b
Crude palm kernel oil (CPKO)	37.51±11.91	17.50±8.94	22.86±4.01 ^a	37.28±9.99 ^a	30.80±8.25 ^a
SEM	0.468	0.350	0.322	0.285	0.235
P-value					
Factor A	0.000	0.000	0.000	0.000	0.000
Factor B	0.107	0.152	0.000	0.000	0.000
Factor A x B	0.000	0.059	0.000	0.002	0.006

^{a-g} Means with different superscript letters within the same column are significantly different ($P \leq 0.05$) and ($P \leq 0.01$)

^{ns} Means with the same superscript letters within the same column are not significantly different ($P > 0.05$)

Total Phenolic Content (TPC) (mg GAE/g DW); Total Antioxidant capacity (TAC) (mg GAE/g DW); DPPH (1,1-diphenyl-2-picrylhydrazyl) (DPPH assay) (%); Trolox equivalent antioxidant capacity (TEAC) using (ABTS assay) (2,2'-casino-bis-3-ethylbenzthiazoline-6-sulphonic acid) (%); Ferric Ion Reducing Antioxidant Power assay (FRAP Assay) (%)



Hemp leaves (A)

Hemp root (B)

Figure 1. The phytochemical composition of hemp leaves and roots is revealed through spectral analysis using High-Performance Liquid Chromatography (HPLC)

The investigation of the ratio of hemp leaves to CPKO (6%, 12%, and 18% w/v) and the duration of heating duration for activation of decarboxylation (0, 2, 4, 6, and 8 h) revealed a significant interaction between the hemp leaves proportion and heating duration ($P < 0.01$), as shown in Table 3. Specifically, the 12% hemp leaves to CPKO proportion significantly resulted in a higher TPC compared to the 6% and 18% ratios ($P < 0.01$). Additionally, prolonged heating duration for 8 hours led to TPC levels that exceeded those observed at shorter durations (0, 2, 4, and 6 h) ($P < 0.01$). and the interaction of 12% hemp leaves with CPKO and heated time for 6 hours produced significantly higher TPC compared with other interactions. ($P < 0.01$) (Figure 2A).

In terms of total antioxidant capacity (TAC), the 6% hemp leaves to CPKO proportion exhibited significantly higher values compared to the 12% and 18% proportions ($P < 0.01$), and heating time of 4 h also demonstrated increased TAC compared to other durations (0, 2, 6 and 8) ($P < 0.01$). However, the interaction of the hemp leaves ratio and 6% hemp leaves to CPKO with heating duration for 4 h gave a higher TAC than other interactions ($P = 0.007$) (Figure 2B). The results of the antioxidant activity assessed using DPPH, ABTS, and FRAP assays demonstrated that the 12% hemp leaves to CPKO ratio significantly outperformed the other proportions (6% and 3%) ($P < 0.01$). The influence of heating duration on the reaction of decarboxylation, particularly at 4 and 6 h. showed superior results compared to other time points (0, 2, and 8 h) ($P < 0.01$).

In terms of the interaction between hemp leaves proportions and heating durations, the combination of 6% hemp leaves with 6 h of heating duration, yielded the highest DPPH activity in all tested interactions ($P < 0.01$) (Figure 2C). Similarly, heating durations of 4 and 6 h resulted in the highest antioxidant activity

in both the ABTS and FRAP assays in all tested interactions ($P < 0.01$) (Figures 2D and E).

Table 3. Effect of decarboxylation duration and the ratio of hemp and medium-chain fatty acid triglyceride oil on total phenolic content (TPC) and antioxidant capacity of hemp leaves extract

Factor	TPC mg GAE/g DW	TAC mg GAE/g DW	DPPH assay (%)	ABTS assay (%)	FRAP Assay (%)
Interaction between factor A (w/v) x B					
6% × 0 hr	40.24±0.05 ^j	21.16±0.26 ^{bcdef}	26.94±1.00 ^{fg}	32.17±0.76 ⁱ	32.56±1.33 ^g
6% × 2 hr	46.49±0.33 ^h	23.13±1.05 ^{abcd}	27.13±1.11 ^{efg}	40.00±2.00 ^g	34.19±0.13 ^g
6% × 4 hr	47.32±0.15 ^g	25.45±0.74 ^a	26.42±0.58 ^g	44.52±0.50 ^{ef}	38.42±0.31 ^f
6% × 6 hr	47.08±0.07 ^h	24.62±2.51 ^{ab}	26.44±0.48 ^g	46.33±3.21 ^e	39.61±0.38 ^{ef}
6% × 8 hr	56.83±0.16 ^{abc}	21.60±1.64 ^{bcde}	26.22±0.35 ^g	50.20±0.91 ^d	40.40±1.03 ^{de}
12% × 0 hr	41.04±0.06 ^{ij}	20.02±1.16 ^{cdefg}	28.36±0.19 ^{defg}	35.00±1.00 ^{hi}	42.38±1.11 ^d
12% × 2 hr	55.84±0.16 ^{bcd}	20.37±1.43 ^{cdefg}	29.46±0.47 ^{cdef}	51.17±0.85 ^{cd}	49.33±0.80 ^b
12% × 4 hr	54.14±0.11 ^e	23.80±1.06 ^{abc}	31.63±0.78 ^{bc}	62.13±1.03 ^a	52.56±0.52 ^a
12% × 6 hr	57.38±0.45 ^a	21.55±1.34 ^{bcde}	34.43±1.74 ^a	64.33±1.53 ^a	53.18±2.03 ^a
12% × 8 hr	56.87±0.96 ^{ab}	21.35±0.57 ^{bcde}	33.35±0.95 ^{ab}	58.07±0.90 ^b	49.86±0.94 ^b
18% × 0 hr	42.02±0.49 ⁱ	19.22±1.08 ^{efg}	29.62±0.92 ^{cde}	36.20±0.35 ^h	42.07±0.64 ^d
8% × 2 hr	52.80±0.68 ^f	19.67±1.15 ^{defg}	31.20±0.75 ^{bc}	54.53±0.50 ^{bc}	50.50±0.66 ^b
18% × 4 hr	55.62±0.24 ^{cd}	18.67±1.15 ^{efg}	30.53±0.92 ^{cd}	57.27±0.64 ^b	51.26±0.67 ^{ab}
18% × 6 hr	55.07±0.53 ^{de}	17.35±1.44 ^{fg}	29.49±0.53 ^{cde}	55.63±0.55 ^b	50.45±1.21 ^b
18% × 8 hr	55.10±0.04 ^{bc}	17.20±1.14 ^g	29.28±0.63 ^{cdef}	42.20±0.40 ^{fg}	47.31±0.72 ^c
Factor A: Proportion of hemp leaves to Crude palm kernel oil (Leaves: CPKO)					
6% w/v	47.80±5.50 ^c	23.23±2.16 ^a	26.64±0.74 ^c	42.64±6.57 ^c	37.02±3.25 ^c
12% w/v	53.38±6.57 ^a	21.42±1.68 ^b	31.44±2.50 ^a	54.14±10.97 ^a	49.46±4.08 ^a
18% w/v	52.12±5.34 ^b	18.42±1.44 ^c	30.02±0.99 ^b	49.17±8.70 ^b	48.32±3.57 ^b
Factor B: Decarboxylation reaction time (Heating duration)					
0 hr	41.10±0.81 ^e	20.13±1.17 ^b	28.31±1.35 ^b	34.45±1.91 ^d	39.00±4.86 ^d
2 hr	51.71±4.15 ^b	21.05±1.91 ^b	29.26±1.90 ^{ab}	48.57±6.68 ^c	44.67±7.78 ^c
4 hr	52.70±3.34 ^c	22.71±3.25 ^a	29.55±2.44 ^a	54.64±7.90 ^a	47.38±6.73 ^a
6 hr	53.18±4.69 ^b	21.17±3.54 ^b	30.12±3.61 ^a	55.43±8.00 ^a	47.75±6.25 ^a
8 hr	56.81±1.50 ^a	20.05±2.37 ^b	29.61±3.15 ^a	50.15±6.90 ^b	45.86±4.25 ^b
SEM	0.234	0.739	0.487	0.718	0.301
P-value					
Factor A	0.000	0.000	0.000	0.000	0.000
Factor B	0.000	0.001	0.002	0.000	0.000
Factor A x B	0.000	0.007	0.000	0.000	0.000

^{a-g} Means with different superscript letters within the same column are significantly different ($p \leq 0.05$) and ($p \leq 0.01$)

^{ns} Means with the same superscript letters within the same column are not significantly different ($p > 0.05$)
 Total Phenolic Content (TPC) (mg GAE/g DW); Total Antioxidant capacity (TAC) (mg GAE/g DW); DPPH (1,1-diphenyl-2-picrylhydrazyl) (DPPH assay) (%); Trolox equivalent antioxidant capacity (TEAC) using (ABTS assay) (2,2'-casino-bis-3-ethylbenzthiazoline-6-sulphonic acid) (%); Ferric Ion Reducing Antioxidant Power assay (FRAP Assay) (%)

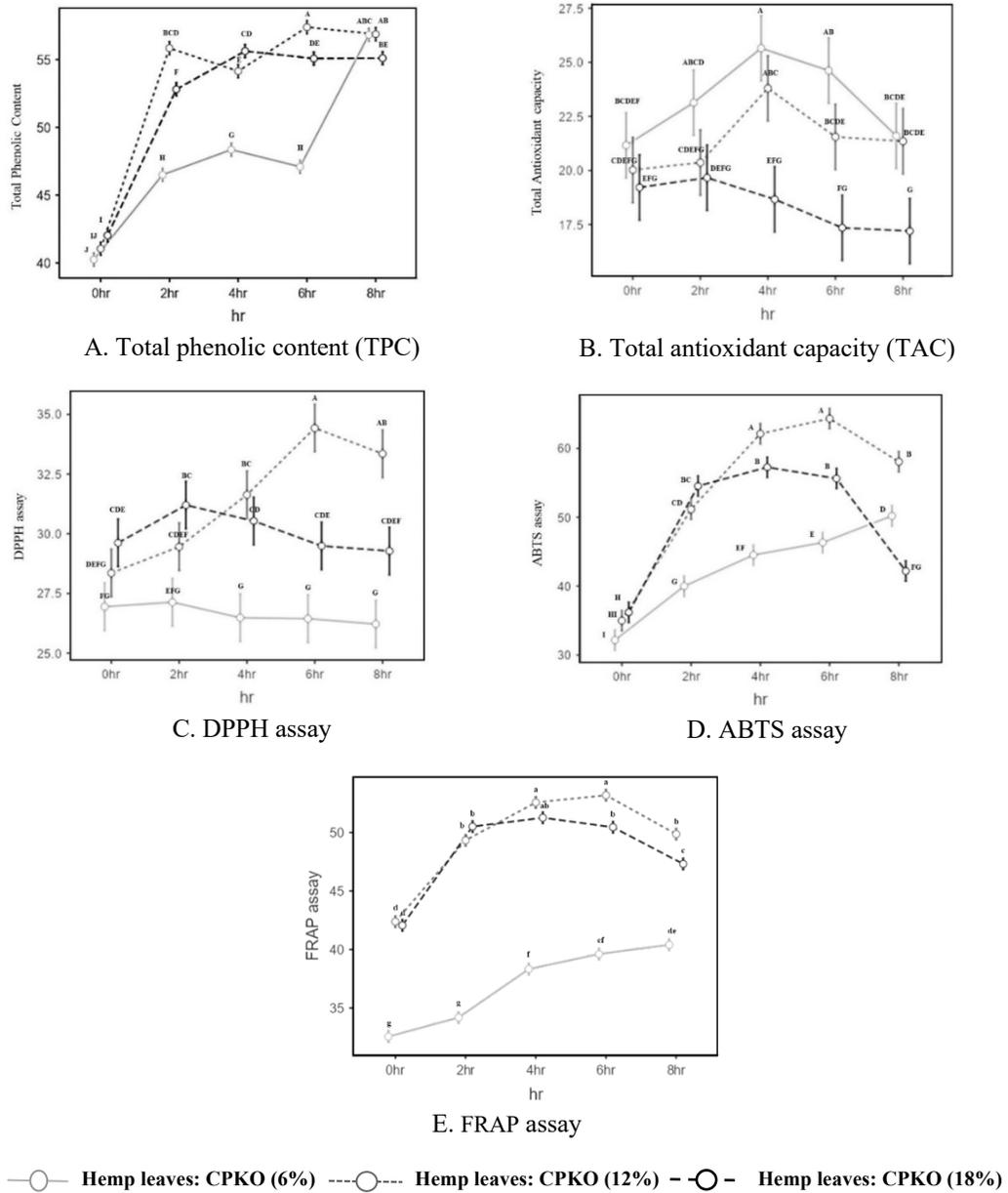


Figure 3. Schematic diagram showing the changes of total phenolic content, total antioxidant capacity, DPPH assay, ABTS assay, and FRAP assay concerning time (0, 2, 4, 6, and 8 h) upon heating for inducing decarboxylation to extract phytocannabinoids from hemp leaves

An analysis of the cannabinoid content resulting from the interaction between 12% hemp leaves with heating durations of 4 and 6 h demonstrated that decarboxylation-enhanced phytocannabinoid extraction significantly increased CBD, CBDA, and total CBD concentrations after 6 hours of heating. Specifically, concentrations were recorded at 0.004, 0.005, and 0.008, representing corresponding 33.33%, 50%, and 38% increases compared to the 4-h heating duration (Figure 4). The fatty acid profile revealed an increase in saturated fatty acids by 1.45 g/100 g. In contrast, levels of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids, and total unsaturated fats including Omega-3, Omega-6, and Omega-9 decreased by 1.95 g/100 g, 0.92 g/100 g, 2.88 g/100 g, 1.07 mg/100 g, 926.95 mg/100 g, and 1,950.72 mg/100 g, respectively (Table 4). Regarding the chemical composition, the energy value for the mixture of 12% hemp leaves and CPKO heated for 6 h was found to be 881.32 kcal/100 g, a 7.11 kcal/100 g increase compared to CPKO alone. Significantly, both the heavy metal content and total plate count of CPKO and the phytocannabinoid oil extract (12% hemp leaves: CPKO heated for 6 hours) met safety standards, confirming its suitability to be applied as an energy source or livestock feed additive (Table 6).

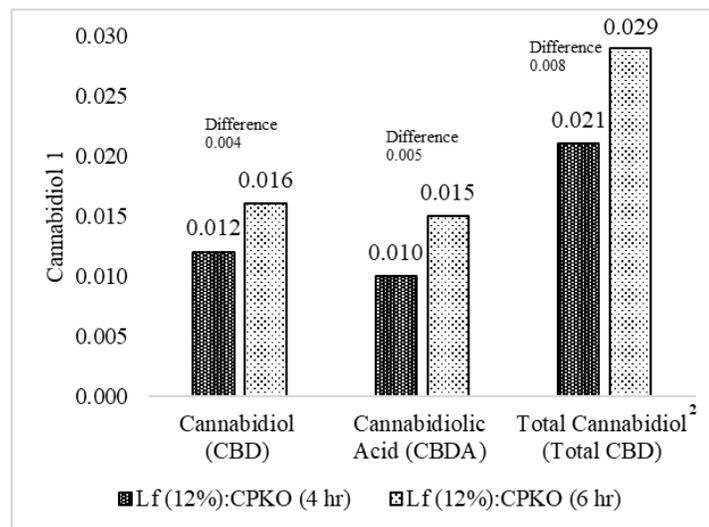


Figure 4. A comparative diagram of the cannabinoid content in oils, comparing the proportion of hemp leaves to 12% CPKO and the heating duration of 4 and 6 h

¹Cannabidiol test: In-house method based on Journal of AOAC Official Method 2018.11, Revised First Action 2020 P.1,822-1,833 with Cannalysis siam herbal laboratories. ²Total CBD = %CBD + (%CBDA*0.877)

Table 4. Fatty acid composition of crude palm kernel oil and phytocannabinoid oil extracts as raw materials for prototype energy supplement products for livestock

Fatty acid composition ^{1/} (g/100g)	CPKO	CPKO oil contained ^{2/} phytocannabinoid extract
Caproic acid (C6:0)	0.23	0.26
Caprylic acid (C8:0)	3.43	3.67
Capric acid (C10:0)	3.29	3.48
Lauric acid (C12:0)	45.50	47.09
Medium-chain fatty acids (MCFA)	52.45	54.50
Myristic acid (C14:0)	15.86	15.86
Palmitic acid (C16:0)	8.64	8.22
Stearic acid (C18:0)	2.36	3.24
Arachidic acid (C20:0)	0.12	2.19
Behenic acid (C22:0)	0.03	0.03
Lignoceric acid (C24:0)	0.05	0.05
Saturated Fatty acid (g/100g)	79.68	81.10
Palmitoleic acid (C16:1n7)	0.02	0.02
Trans-9-Elaidic acid (C18:1n9-t)	0.03	0.04
cis-9-Oleic acid (C18:1n9-c)	15.55	13.6
cis-11-Eicosenoic acid(C20:1n11-c)	0.10	0.02
Nervonic acid (C24:1n9)	0.04	0.05
Monounsaturated fatty acid (g/100g)	15.74	13.79
cis-9,12-Linoleic acid (C18:2n6)	2.47	1.55
cis-11,14-Eicosadienoic acid (C20:2)	0.01	0.02
Arachidonic acid (C20:4n6)	0.02	0.02
Polyunsaturated Fatty acid (g/100g)	2.51	1.59
Unsaturated fat (g/100g)	18.26	15.38
Omega 3 (mg/100g)	6.53	5.46
Omega 6 (mg/100g)	2,493.04	1,566.09
Omega 9 (mg/100g)	15,594.87	13,644.15

^{1/} Fatty acid composition using In-house method TE-CH-208 based on AOAC. (2019)

^{2/} CPKO oil contained phytocannabinoid extract is 12% hemp leaves: CPKO & 6 hr heating duration.

Emulsion of medium-chain fatty acids with 40% lauric acid plus phytocannabinoids (EMPL): A prototype energy supplement for suckling piglets. The EMPL product offers an energy value of 788.94 kcal per 100 g, and a fat content of 87.66 g per 100 g. and water activity (Aw) is 23.38%. Key chemical properties include an iodine value (IV) of 41.89% and a peroxide value (PV) of 4.73 mEq peroxide/kg. The fatty acid profile (g/100 g) is as follows: lauric acid (C12:0), medium-chain fatty acids (MCFAs), myristic acid (C14:0), saturated fatty acids, oleic acid (C18:1n9t), monounsaturated fatty acids, polyunsaturated

fatty acids, and total unsaturated fats, with respective values of 42.21, 44.62, 5.93, 61.04, 15.92, 16.02, 9.17, and 25.12. Omega-3, -6, and -9 fatty acids are present in concentrations of 586.15 mg, 5,862.10 mg, and 15,970.20 mg per 100 g, respectively.

Table 5. Chemical composition, heavy metal test, and total plate count of crude palm kernel oil (CPKO) and CPKO contained phytocannabinoid oil extracts

Items ^{1/}	Oil type	
	CPKO	CPKO oil is contained. phytocannabinoid extract
Chemical composition		
Ash ^{2/} (g/100g)	<0.01	0.04
Calories from Fat ^{3/} (kcal/100g)	888.42	881.32
Carbohydrate ^{3/} (g/100g)	2.19	3.21
Fat ^{2/} (g/100g)	97.74	96.48
Water Content ^{5/} (%)	0.07	0.27
Iodine Value ^{6/} (%)	16.89	10.53
Peroxide Value ^{7/} (mEq Peroxide/kg)	3.85	2.87
Heavy Metal Test (mg/kg)		
Arsenic (As) ^{8/}	ND	ND
Cadmium (Cd) ^{8/}	ND	ND
Lead (Pb) ^{8/}	<0.050	0.17
Mercury (Hg) ^{9/}	ND	ND
Total plate count		
<i>Salmonella spp.</i> ^{10/} (in 25g)	ND	ND
Total Plate Count ^{11/} (CFU/g)	<10	<10
Yeast and Molds ^{12/} (CFU/g)	<10	<10

^{1/}Central Laboratory (Thailand) Co., Ltd. ^{2/} Ash: AOAC Method 942.05 (AOAC, 2012) ^{3/} Calories (Fat and Carbohydrate): In-house method TE-CH-169 (Central Laboratory, 1993), adapted from Method of Analysis for Nutrition Labelling (p. 106). ^{4/} Fat: AOAC Method 920.39 (AOAC, 2023) ^{5/} Water Content: AOAC Method 934.01. (AOAC, 2005) ^{6/} Iodine Value: In-house method (Central Laboratory) based on AOCS Cd 1-25 (AOCS, 2017). ^{7/} Peroxide Value: In-house method (Central Laboratory) based on AOCS Cd 8-53 (AOCS, 2003). ^{8/} Arsenic, Cadmium, Lead: In-house method TE-CH-260 (Central Laboratory) with AOAC Methods 2013.06 & 999.10 (AOAC, 2019). ^{9/} Mercury: In-house method TE-CH-260 (Central Laboratory) with AOAC Method 2013.06 (AOAC International, 2019). ^{10/} *Salmonella spp.*: ISO 6579-1:2017/Amd. 1:2020. ^{11/} Total Plate Count: In-house method (Central Laboratory) based on FDA BAM Online (2001, Chapter 3). ^{12/} Yeast and Molds: In-house method (Central Laboratory) based on FDA BAM Online (2001, Chapter 18).

This study aimed to assess the effects of a novel energy supplement prototype (EMPL) for suckling piglets in a commercial production environment. Piglets were orally administered 3.5 ml of EMPL per piglet. The first time was administered within 12–24 h after birth, and the second was administered on the

third day post-parturition. The results were compared to those of a control group that received the antibiotic toltrazuril. It was found that piglets in the EMPL group exhibited significantly higher body weight on the fifth day than the control and antibiotic groups ($P < 0.01$). Moreover, the average daily gain (ADG) of the EMPL group exceeded that of the control by 30.61% ($P < 0.01$) (Table 6), indicating enhanced growth performance of piglets.

Table 6. Effects of oral administration of EMPL in suckling piglets compared with the control group (antibiotics) on productive performance

Parameter	Control (CON)	EMPL40	Pr(t)
Piglet (1.00 - 1.50 kg)	35	35	
Initial weight (kg)	1.27±0.02	1.31±0.02	0.124
Weight 24 hr (kg)	1.38±0.02	1.43±0.02	0.149
ADG 24 hr (g/day)	115.00±8.66	118.25±6.43	0.764
Colostrum intake (ml/piglet)	314.34±11.46	319.87±8.71	0.702
Weight at 5 th d (kg)	1.73±0.05	2.00±0.05	<0.001
ADG at 5 th d (g/day)	92.00±6.25	132.59±4.72	<0.001
Milk intake at 5 th d (ml/piglet)	468.31±12.88	552.01±11.33	<0.001

Discussion

Hemp leaves and roots, often considered byproducts of cultivation, are traditionally used for extracting phytocannabinoids through oil-based solvent heating. Cold-pressed coconut oil or medium-chain triglycerides (MCTs) are commonly employed as solvents, leveraging the medicinal properties of the extracts in traditional Thai medicine (Maly *et al.*, 2023). The incorporation of hemp extract in oil form presents significant potential as a feed additive for animals, particularly in enhancing immune function, reducing inflammation, promoting intestinal health, and alleviating stress in pets. This approach aligns with the objectives of sustainable animal husbandry by reducing reliance on antibiotics and chemical treatments (Atalay *et al.*, 2020; Iffland and Grotenhermen, 2017). Hemp-derived phytocannabinoids, including cannabidiol (CBD), exhibit therapeutic effects such as anti-inflammatory and immunomodulating properties. Tetrahydrocannabinol (THC), present in trace amounts (<0.3%), is associated with appetite stimulation and pain relief (Carcieri *et al.*, 2018). Cannabigerol (CBG) shows antibacterial and neuroprotective properties, while Cannabichromene (CBC) and Cannabinol (CBN) offer analgesic and anti-inflammatory benefits (Borrelli *et al.*, 2013; Khouchlaa *et al.*, 2024). The mechanism of the decarboxylation process, reported by Fućak *et al.* (2023); and Moreno *et al.* (2020) found that CBD is a pharmacologically active compound, is derived from CBDA through decarboxylation, a process that activates the β -keto

acid pathway, cleaving the C–C bond and releasing CO₂, thereby converting cannabinoids into their active forms.

The comparative phytochemical and nutritional analysis of hemp leaves and roots revealed an inverse relationship in cannabinoid concentrations: roots exhibited higher levels of CBDA, whereas leaves were richer in protein and energy. The phytochemical and bioactive profiles outlined in Table 1 provide valuable insights for optimizing the utilization of the entire hemp plant. Additionally, Moreno *et al.* (2020) demonstrated that alcoholic solvents enhanced the extraction efficiency of hemp roots compared to CPKO, as shown in Experiment 1 (Table 2). This outcome can be attributed to the heat-dissipating properties of CPKO, which preserve the integrity of active compounds. Two critical factors must be considered in the extraction of phytocannabinoids from hemp using medium-chain triglyceride (MCT) oil; first is the decarboxylation process, in which heat or pressure causes the removal of the carboxyl group and CO₂, typically at temperatures between 110°C and 130°C for 4 to 6 h. The second is the application of high pressure, ranging from 1.5 to 2.0 MPa, in a process known as "autoclave sterilization". This method ensures sterility and enhances decarboxylation efficiency by breaking down cell structures, thereby improving compound extraction (Lohmann *et al.*, 2019).

However, it is important to note that inappropriate temperatures can lead to the degradation of some effective compounds. Therefore, optimizing the temperature and duration is essential for activating raw materials before the extraction process (Da Porto *et al.*, 2014). Another significant consideration is the use of MCT oil as a solvent for extracting phytocannabinoids from hemp leaves. MCT oil acts as a stable carrier, enhancing the bioavailability and absorption of cannabinoids. To ensure the purity of the extract, incorporating a filtration step to eliminate residual hemp material is critical. For this purpose, it is advisable to use cloth bags or nylon mesh to enclose the herbal materials during the extraction process. (Reason *et al.*, 2022; Nguyen *et al.*, 2024). Consequently, MCT oil emerges as an effective solvent for facilitating phytocannabinoid extraction. This finding aligns with the research of Brunel *et al.* (2011), who highlights the potential of medium-chain fatty acid triglyceride oils, such as palm kernel oil (PKO) and coconut palm kernel oil (CPKO), in decarboxylation reactions, suggesting promising applications in the food industry and the production of additives in the future.

Although hemp roots contain notable amounts of CBDA, the conversion of CBDA into its active form, CBD, indicates that the antioxidant content and activity are significantly higher in hemp leaf extracts than root extracts. This observation aligns with the findings of Gul *et al.* (2021) and Moreno *et al.* (2020). However, hemp roots are rich in distinct compounds, such as alkaloids and

triterpenoids. The lower hydroxyl group content and unique bonding patterns in hemp roots, relative to the leaves, may explain their reduced antioxidant potential. Hemp leaves possess a greater abundance of hydroxyl groups (-OH) attached to aromatic rings, which greatly enhance their antioxidant capacity. These compounds are more chemically reactive, particularly during decarboxylation, compared to the more stable structures found in hemp roots. Kornpointner *et al.* (2021) observed that phytocannabinoid levels in hemp roots are either absent or present in minimal quantities, thus contributing to their distinct applications. However, using alcohol as the extraction solvent yielded the highest concentrations of friedelin and epifriedelinol, with values ranging from 0.205 mg/g DW to 0.059 mg/g DW. Similarly, Jin *et al.* (2020) reported that air-dried hemp roots analyzed after 24 h showed a CBD ratio of 1:2. In contrast, freeze-dried roots consistently exhibited higher epifriedelinol content compared to those dried using heat-based methods. They also suggested that a reduction in friedelin and epifriedelinol content may be due to drying temperatures exceeding 45°C for more than 30 minutes, as well as extended post-harvest drying. This finding implies that inappropriate drying temperatures and prolonged storage negatively impact triterpenoid levels. However, no significant differences were found between ethanol, hexane, and CO₂ extraction methods compared to conventional triterpenoid extraction techniques. Nevertheless, ethanol proved to be the most efficient and environmentally sustainable option for extracting friedelin and epifriedelinol from hemp roots. In addition to phytocannabinoids, hemp leaves are rich in other bioactive compounds, such as terpenes, including myrcene, limonene, pinene, and linalool, which are known for their anti-inflammatory and antimicrobial effects. The terpene concentration in hemp leaves is higher than in roots, as leaves produce terpenes primarily for defense against herbivores and insects. In contrast, the roots generate lower terpene levels, focusing on protection from soil-borne fungi and bacteria (Booth *et al.*, 2020; Tomar *et al.*, 2021). Flavonoids, including quercetin, kaempferol, and apigenin, act as potent antioxidants, aiding in the reduction of inflammation and protection of cells from oxidative stress. These flavonoids are more abundant in leaves, as they serve photosynthetic functions, whereas roots, though also containing flavonoids, utilize them mainly for defending against soil microorganisms (Palazzolo *et al.*, 2020; Farag and Kayser, 2015). This observation aligns with the phytochemical analyses that compared hemp leaves and roots, as illustrated in Table 1.

Decarboxylation is a key process in phytocannabinoid extraction from hemp, converting inactive cannabinoid acids into biologically active forms, notably CBD and THC. These cannabinoids are valuable for livestock energy supplements, particularly for piglets, which require high energy for growth and health. Citti *et al.* (2018) showed how this process transforms CBDA and THCA

into CBD and THC. Lima *et al.* (2022) found that activated cannabinoids support metabolism, reduce stress, and enhance immune function in animals. Integrating phytocannabinoids with fats or medium-chain fatty acids into energy supplements provides absorbable energy, promoting piglet growth and mitigating oxidative stress (Miranda-Cortes *et al.*, 2023). The factors influencing the decarboxylation of hemp leaves and roots highlight the solvent role of CPKO. Hemp leaves exhibited higher TPC and antioxidant capacity compared to roots, attributed to the removal of carboxyl groups (-COOH) from acidic phenolics during decarboxylation. This process in leaves enhances neutral cannabinoids and reactive phenolic hydroxyl groups, increasing antioxidant reactivity through potential hydrogen bond formation. In contrast, hemp roots, with simpler polyphenolic structures and fewer conjugated double bonds, show minimal transformation during decarboxylation, maintaining structural stability (Moreno *et al.*, 2020).

The phenolic compounds in hemp roots exhibit greater stability compared to the more reactive phenolics found in hemp leaves. According to Gul *et al.* (2021), while hemp root extracts contain lower levels of polyphenols than those derived from leaves, they provide unique bioactive components, including alkaloids and triterpenoids. The structural simplicity of the compounds in hemp roots—characterized by fewer hydroxyl groups and distinct bonding patterns—plays a key role in their resistance to alterations caused by decarboxylation. In contrast, the phenolic compounds in hemp leaves, such as flavonoids and lignans, have a higher abundance of hydroxyl (-OH) groups attached to aromatic rings. This feature not only enhances their antioxidant properties but also increases their structural complexity. The positioning of hydroxyl groups at the ortho or para sites in flavonoid molecules further facilitates radical scavenging, which explains the superior antioxidant activity of hemp leaf extracts. These characteristics make hemp leaves particularly suitable for phytocannabinoid extraction due to their intricate phenolic structures and enhanced hydrogen-bonding potential.

The control factors for raw material preparation, such as drying and grinding, before the activation process, are critical steps in optimizing extraction efficiency. The consistency of these findings with those of Olmos *et al.* (2022) suggested that slow drying methods for raw material preparation (Challa *et al.*, 2021) and particle size reduction to less than 0.5 mm for milling or sieving (Ko and Hughes, 2019) are essential steps before solvent extraction. This study determined that a ratio of 12% hemp leaves to CPKO was optimal, corroborating the work of Carcieri *et al.* (2017), who reported a 10% ratio for cannabis flower extraction using olive oil. During extraction, the packing method of hemp leaves was given particular attention to the packaging method, in which cloth bags were employed to contain hemp leaves, facilitating efficient waste filtration and

sterilization. This approach enhances material distribution and sterilization, consistent with the findings of Olmos *et al.* (2022). The activation of raw materials is a critical step in the extraction process, and various methods, such as prolonged low-temperature heating or ultrasonic stimulation, can promote decarboxylation. In this experiment, raw materials were sterilized at 121°C for 21 minutes in an autoclave, ensuring effective activation under controlled pressure and temperature. Both the raw materials and the cloth bags underwent sterilization before the activation process. The study found a statistically significant interaction ($P < 0.01$) between the proportion of hemp leaves to CPKO and the heating duration on extraction outcomes. A ratio of 12% hemp leaves to CPKO, heated at 110°C for 4-6 h, produced the highest antioxidant content and activity, supporting the findings of Fućak *et al.* (2023). The optimal temperature range for extraction was determined to be between 110°C and 120°C, with activation times of 2 to 6 h in a hot air oven. Specifically, heating at 110°C for 6 h significantly enhanced the decarboxylation process, increasing the total CBD content in hemp leaves by more than 38% compared to the 4 h heating duration.

The prototype product, EMPL, containing 25-29% phytocannabinoid extract in CPKO and formulated to include concentrations of 40% lauric acid, was designed for oral administration. Which is an effective method of delivering liquid nutrients to suckling piglets, allowing for precise control over nutrient and energy intake. This approach particularly benefits a sow's inadequate production or weak piglets after birth (Jackman *et al.*, 2020). Phytocannabinoids have been shown to reduce stress by interacting with the endocannabinoid system, thereby helping to regulate stress responses (Hassan *et al.*, 2023). Additionally, their anti-inflammatory properties support gut health, reduce inflammation, improve nutrient absorption, and promote growth performance (Gyires & Zádori, 2016; Toschi *et al.*, 2020). A study by Lauridsen *et al.* (2020) observed that MCFAs, along with other energy-dense liquids, promote rapid energy uptake, facilitating accelerated growth in piglets during the suckling phase. The antimicrobial and energy-rich properties of lauric acid and phytocannabinoids further enhance piglet vitality. Vodolazska *et al.* (2023) also highlighted improvements in intestinal barrier integrity and microbiota modulation, leading to better health outcomes. This increased energy availability encourages "udder-sitting" behavior, where piglets remain close to the udder to optimize colostrum and milk intake, supporting stronger growth and development. The aforementioned findings support the hypothesis that EMPL, as an energy supplement, promotes growth performance in piglets by providing readily accessible energy. Moreover, piglets receiving EMPL exhibited improved suckling behavior, as evidenced by a significant increase in milk intake ($P < 0.01$). This suggests that EMPL may

enhance piglet vitality and responsiveness, improving their ability to stimulate and maintain access to the sow's teats, ultimately increasing milk intake.

An evaluation of EMPL in suckling piglets demonstrated positive effects on growth performance and improved production efficiency. To further elucidate its impact, a comprehensive study of the blood parameters in piglets is recommended.

Phytocannabinoids were extracted from 12% hemp leaves in CPKO (crude palm kernel oil) as the solvent. The extraction process included autoclaving at 121°C for 21 minutes under 0.11 MPa to activate the hemp materials, followed by heating in a hot air oven at 110°C for 6 h to facilitate decarboxylation and extraction. The resulting EMPL emulsion was formulated to contain 25-29% phytocannabinoid extract in CPKO, with adjustments made to achieve a 40% lauric acid content by incorporating triglyceride oil and lauric acid powder. Improved milk consumption, weight gain, and growth rates during the suckling period were observed in piglets that were orally administered the EMPL supplement.

Acknowledgments

This work was financially by Naresuan University (NU), and the Thailand Science Research and Innovation (TSRI) Grant No. R2567B039. We also appreciate the support provided by the Medical Cannabis Research Laboratory, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, for facilitating the examination of hemp leaf and root cannabinoid derivatives. Additionally, we acknowledge the supply of raw materials from Firstly Tech Co., Ltd. and Prototype factory producing feed additives in livestock (Agri InNovel Tech) from N Feed Plus Co., Ltd.

References

- Andre, C. M., Hausman, J. F. and Guerriero, G. (2016). *Cannabis sativa* L.: the plant of the thousand and one molecules. *Frontiers in Plant Science*, 7:19.
- Atalay, S., Jarocka-Karpowicz, I. and Skrzydlewska, E. (2020). Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants*, 9:21.
- AOAC (1993). Official methods of analysis of the Association of Official Analytical Chemists: Official methods of analysis of AOAC International (14th ed.). Association of Official Analytical Chemists, USA.
- AOAC (2005). Official methods of analysis of the Association of Official Analytical Chemists: Official methods of analysis of AOAC International (18th ed.). AOAC International. (Method 934.01).
- AOAC (2012). Official methods of analysis of the Association of Official Analytical Chemists: Official methods of analysis of AOAC International (19th ed.). AOAC International. (Method 942.05).
- AOAC (2019) Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington DC.

- AOAC (2023). Official methods of analysis of the Association of Official Analytical Chemists: Official methods of analysis of AOAC International (22nd ed.). AOAC International. (Method 920.39).
- AOCS (2017). Official methods and recommended practices of the American Oil Chemists' Society (7th ed.). AOCS Press. (Method Cd 1-25).
- AOCS (2003). Official methods and recommended practices of the American Oil Chemists' Society (6th ed.). AOCS Press. (Method Cd 8-53).
- Bartončíková, M., Bartončíková, B., Lapčík, L. and Valenta, T. (2023). Hemp-Derived CBD Used in Food and Food Supplements. *Molecules*, 28:8047.
- Benkhaira, N., Koraichi, S. I. and Fikri-Benbrahim, K. (2022). In vitro methods to study antioxidant and some biological activities of essential oils: a review. *Biointerface Research in Applied Chemistry*, 12:3332-3347.
- Booth, J. K., Page, J. E. and Bohlmann, J. (2020). Terpene synthases from *Cannabis sativa*. *PLoS One*, 12:e0173911.
- Borrelli, F., Fasolino, I., Romano, B., Capasso, R., Maiello, F., Coppola, D., Orlando, P., Battista, G., Pagano, E., Di Marzo, V. and Izzo, A. A. (2013). The beneficial effect of the non-psychoactive plant cannabinoid cannabigerol on experimental inflammatory bowel disease. *Biochemical Pharmacology*, 85:1306-1316.
- Brunela, H. P., Buijsa, W., Spronsena, J. V., Roosmalenb, C. J., Verpoorted, R., and Witkampa, G. J. (2011). Decarboxylation of D9-tetrahydrocannabinol: Kinetics and molecular modeling. *Journal of Molecular Structure*, 987:67-73.
- Carcieri, C., Tomasello, C., Simiele, M., De Nicolò, A. and Avataneo, V. (2017). Cannabinoids extraction from *Cannabis sativa L.* using palm kernel oil: Optimization and yield analysis. *Journal of Agricultural and Food Chemistry*, 65(28):5655-5661.
- Challa, S. K. R., Misra, N. N. and Martynenko, A. (2021). Drying of cannabis—state of the practices and future needs. *Drying Technology*, 39:2055-2064.
- Carcieri, C., Tomasello, C., Simiele, M., Di Nicolò, A., Avataneo, V., Canzoneri, L., Cusato, J., Di Perri, G. and D'Avolio, A. (2018). Cannabinoid concentration variability in cannabis olive oil galenic preparations. *Journal of Pharmacy and Pharmacology*, 70:143-149.
- Citti, C., Braghiroli, D., Vandelli, A. M. and Cannazza, G. (2018). Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *Journal of Pharmaceutical and Biomedical Analysis*, 147:565-579.
- Da Porto, C., Decorti, D. and Natolino, A. (2014). Microwave pretreatment of hemp (*Cannabis sativa L.*) seed: Optimization of oil yield and quality. *Journal of Agricultural and Food Chemistry*, 62:9651-9655.
- Farag, S. and Kayser, O. (2015). Cannabinoid production by hairy root cultures of *Cannabis sativa L.* compared to cell suspension cultures. *Natural Product Communications*, 10:421-426.
- FDA (2001). Bacteriological Analytical Manual (BAM) online. U.S. Food and Drug Administration. Chapters 3 and 18.
- Fox, J., and Weisberg, S. (2020). Car: Companion to Applied Regression. [R package]. Retrieved from <https://cran.r-project.org/package=car>.
- Fučák, T., Kreft, S., Svedružić, Z. M. and Tavčar, E. (2023). Mechanism and kinetics of CBDA decarboxylation into CBD in hemp. *Journal of Plant Biochemistry and Biotechnology*, 32:608-621.
- Gul, S., Gul, H., Masoodi, F. A., Shah, Z. A. and Ganai, S. A. (2021). Phytochemical and pharmacological review of *Cannabis sativa L.* with a special focus on industrial hemp. *Journal of Phytomedicine*, 10:189-204.
- Gyires, K. and Zádori, Z. (2016). Role of cannabinoids in gastrointestinal mucosal defense and inflammation. *Current Neuropharmacology*, 14:935-951.

- Hassan, F., Lin, C., Mehboob, M., Bilal, R. M., Arain, M. A., Siddique, F., Chen, F., Li, Y., Zhang, J., Shi, P., Lv, B. and Lin, Q. (2023). The potential of dietary hemp and cannabinoids to modulate the immune response to enhance health and performance in animals: opportunities and challenges. *Frontiers in Immunology*, 14:1285052.
- Iffland, K. and Grotenhermen, F. (2017). An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. *Cannabis and Cannabinoid Research*, 2:139-154.
- ISO. (2020). Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella (ISO 6579-1:2017/Amd.1:2020). International Organization for Standardization.
- Jackman, J. A., Boyd, J. R. and Elrod, C. C. (2020). Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. *Journal of Animal Science and Biotechnology*, 11:44.
- Jadhav, H. B. and Annature, U. S. (2023). Triglycerides of medium-chain fatty acids: a concise review. *Journal of Food Science and Technology*, 60:2143-2152.
- Jin, D., Dai, K., Xie, Z. and Chen, J. (2020). Secondary metabolites profiled in cannabis inflorescences, leaves, stem barks, and roots for medicinal purposes. *Scientific Reports UK*, 10:1-14.
- Khouchlaa, A., Khourib, S., Hajibe, A., Zeoukd, I., Amaliche, S., Msairif, S., Menyiyg, N., Raish, C., Lahyaouii, M., Khalidj, A., Omarii, N., Kumariq, Y. and Bouyahya, A. (2024). Health benefits, pharmacological properties, and metabolism of cannabinol: A comprehensive review. *Industrial Crops and Products*, 213:118359.
- Ko, R. D. and Hughes, B. (2019). Cannabinoid extraction and distillation. U.S. Patent No. 10,413,843. Washington, DC: U.S. Patent and Trademark Office.
- Kornpointner, C., Martinez, A.S., Marinovic, S., Haselmair-Gosch, C., Jamnik, P., Schroder, K., Lofke, C. and Halbwirth, H. (2021). Chemical composition and antioxidant potential of *Cannabis sativa* L. roots. *Industrial Crops and Products*, 165:113422.
- Lauridsen, C. (2020). Effects of dietary fatty acids on gut health and function of pigs pre- and post-weaning. *Journal of Animal Science*, 98:skaa086.
- Lima, T. M., Santiago, N. R., Alves, E. C. R., Chaves, D. S. A. and Visacri, M. B. (2022). Use of cannabis in the treatment of animals: a systematic review of randomized clinical trials. *Animal Health Research Reviews*, 23:25-38.
- Lohmann, A., Cogan, T. M. and Linstrom, B. (2019). Thermal decarboxylation of cannabinoids: optimization of process parameters using autoclave-based extraction. *Journal of Natural Products*, 82:1241-1249.
- Lust, C. A. C., Hillyer, L. M., Pallister, M., Rogers, M. A., Rock, E. M., Limebeer, C. L., Parker, L. A. and Ma, D. W. L. (2023). Orally consumed cannabinoids: the effect of carrier oil on acute tissue distribution in male C57BL/6 mice. *Research Square*, Retrieved from <https://assets-eu.researchsquare.com/files/rs-4783415/v1/66b42e8d-bcf7-4910-9d86-095ea09840fa.pdf>
- Maly, P., Sutaneg, T. and Kaewjinda, W. (2023). Traditional extraction of phytocannabinoids from hemp byproducts using oil-based solvents: Applications in Thai herbal medicine. *International Journal of Agricultural Technology*, 19:1125-1140.
- Minarti, M., Ariani, N., Megawati, M., Hidayat, A., Hendra, M., Primahana, G. and Darmawan, A. (2024). Potential antioxidant activity methods: DPPH, ABTS, FRAP, total phenol and total flavonoid levels of *Macaranga hypoleuca* (Reichb. f. & Zoll.) leaves extract and fractions. *E3S Web of Conferences*, 503:07005.
- Miranda-Cortés, A., Mota-Rojas, D., Crosignani-Outeda, N., Casas-Alvarado, A., Martínez-Burnes, J., Olmos-Hernández, A., Mora-Medina, P., Verduzco-Mendoza, A. and

- Hernández-Ávalos, I. (2023). The role of cannabinoids in pain modulation in companion animals. *Frontiers in Veterinary Science*, 9:1050884.
- Montero, L., Ballesteros-Vivas, D., Ballesteros-Vivas, A. F. and Sánchez-Camargo, A. D. P. (2023). Hemp seeds: Nutritional value, associated bioactivities and the potential food applications in the Colombian context. *Frontiers in Nutrition*, 9:1039180.
- Moreno, T., Dyer, P. and Tallon, S. (2020). Cannabinoid Decarboxylation: A Comparative Kinetic Study. *Industrial & Engineering Chemistry Research*, 59:20307-20315.
- Moreno-Sanz, G., Vera, C. F., Sánchez-Carnerero, C., Roura, X. N. and Baena, S. D. M. (2020) Biological Activity of *Cannabis sativa* L. Extracts Critically Depends on Solvent Polarity and Decarboxylation. *Separations*, 7:56.
- Nachnani, R., Raup-Konsavage, W. and Vrana, K. E. (2021). The Pharmacological Case for Cannabigerol. *Journal of Pharmacology and Experimental Therapeutics*, 376:204-212.
- Nguyen, N. A., Forstater, H. and McIntosh, J. (2024). Decarboxylation in Natural Products Biosynthesis. *Journal of the American Chemical Society*, 4:2715-2745.
- Olmos, C. L., Chiara-Carcieri, M. T., Hidalgo, J., Ferrerio-Vera, C. and Medina, V. S. (2022). Comprehensive comparison of industrial cannabinoid extraction techniques: Evaluation of the most relevant patents and pilot-scale studies. *Frontiers in Natural Products*, 1:1043147.
- Palazzolo, G., Licata, M., Carbone, A. and Zito, P. (2020). Flavonoids: An overview of their therapeutic potential and recent advances in drug delivery. *Phytotherapy Research*, 34:1838-1856.
- R Core Team (2021). R: A Language and environment for statistical computing. (Version 4.1) [Computer software]. Retrieved from <https://cran.r-project.org>.
- Reason, D. A., Grainger, M. N. C. and Lane, J. R. (2022). Optimization of the Decarboxylation of Cannabis for Commercial Applications. *Industrial & Engineering Chemistry Research*, 61:7823-7832.
- Regan, M., O'Brien, F. and Lynch, P. (2022). Thermal decarboxylation kinetics of cannabinoid acids in hemp biomass: Optimization for industrial processing. *International Journal of Agricultural Technology*, 18:1125-1140.
- Rocca, G. D. and Salvo, A. D. (2020). Hemp in Veterinary Medicine: From Feed to Drug. *Animal Nutrition and Metabolism*, 7:387.
- Ryu, B. R., Islam, M. J., Azad, M. O. K., Go, E. J., Rahman, M. H., Rana, M. S., Lim, Y. S. and Lim, J. D. (2021). Conversion characteristics of some major cannabinoids from hemp (*Cannabis sativa* L.) raw materials by a new rapid simultaneous analysis method. *Molecules*, 26:4113.
- The jamovi project (2022). Jamovi. (Version 2.3) [Computer Software]. Retrieved from <https://www.jamovi.org>.
- Tomar, R. S., Jha, S. K., Singh, V. and Shukla, P. (2021). Cannabinoid biosynthetic pathway: Current and future perspectives in metabolic engineering. *Biotechnology Advances*, 48: 107702.
- Toschi, A., Tugnoli, B., Rossi, B., Piva, A. and Grilli, E. (2020). Thymol modulates the endocannabinoid system and gut chemosensing of weaning pigs. *BMC Veterinary Research*, 16:289.
- Tymoszczyk, M. K. (2013). The effect of oil-soluble rosemary extract, sodium erythorbate, their mixture, and packaging method on the quality of Turkey meatballs. *Journal of food science and technology*, 50:443-454.
- Vaclavik, L., Benes, F., Fenclova, M., Hricko, J., Krmela, A., Svobodova, V., Hajslova, J. and Mastovska, K. (2019). Determination of cannabinoids in cannabis products by liquid chromatography: AOAC Official Method 2018.11. *Journal of AOAC International*, 102:1426-1433.

- Valizadehderakhshan, M., Shahbazi, A., Kazem-Rostami, M., Todd, M. S., Bhowmik, A. and Wang, L. (2021). Extraction of Cannabinoids from *Cannabis sativa* L. (Hemp) - Review. *Agriculture*, 11:384.
- Vodolazska, D. and Lauridsen, C. (2020). Effects of dietary hemp seed oil to sows on fatty acid profiles, nutritional and immune status of piglets. *Journal of Animal Science and Biotechnology*, 11:28.
- Vodolazska, D., Feyera, T., Foldager, L. and Lauridsen, C. (2023). The influence of supplementary feeding during suckling and later weaning on growth performance, incidences of post-weaning diarrhea, and immunity of piglets. *Livestock Science*. 276:105321.

(Received: 2 October 2024, Revised: 24 April 2025, Accepted: 7 May 2025)