

CONJUGATED LINOLENIC ACID CONTENT IN SEEDS OF SOME POT MARIGOLD (*CALENDULA OFFICINALIS* L.) CULTIVARS GROWN IN POLAND

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Abstract

Seed oil of ten cultivars of pot marigold grown in Poland was analyzed in order to determine the main factors affecting its content and composition. The seeds of the pot marigold cultivars grown in Poland contained relatively large amounts of oil ($17.32\% \pm 2.60\%$), which was abundant in conjugated linolenic acids ($49.53\% \pm 5.43\%$) and had low content of saturated fatty acids ($8.62\% \pm 1.97\%$). In addition to the effect of cultivars, the large contents of α -calendic acid in oils were a result of low average temperature and extensive precipitation during seed maturation. The statistical analysis suggested that linoleic acid was the main precursor of α -calendic acid, and α -calendic acid was the precursor of β -calendic acid.

Rezumat

Uleiul din semințele a zece soiuri de gălbenele cultivate în Polonia a fost analizat pentru a determina factorii principali care afectează conținutul și compoziția sa. Semințele soiurilor de gălbenele cultivate în Polonia conțin cantități relativ mari de ulei ($17,32\% \pm 2,60\%$), bogat în acizi linolenici conjugați ($49,53\% \pm 5,43\%$) și are un conținut scăzut de acizi grași saturați. Pe lângă efectul soiurilor, conținutul mare de acid α -calendic în uleiuri este rezultatul temperaturii medii scăzute și al precipitațiilor în cantități masive în timpul maturării seminței. Analiza statistică a sugerat că acidul linoleic a fost principalul precursor al acidului α -calendic, și acidul α -calendic a fost precursorul acidului β -calendic.

Keywords: pot marigold, oil, fatty acid, α -calendic acid, β -calendic acid

Introduction

Pot marigold (*Calendula officinalis* L.), a member of the *Asteraceae* family, is an annual native plant of the Mediterranean area. It has been cultivated as an ornamental species since the ancient times and for medical purposes since the 12th century. For many decades, marigold flower heads (*Calendulae anthodium*) have been known to exhibit spasmolytic, cholagogic, diuretic, anti-inflammatory, antioxidant, and antimicrobial activities [9]. Lately, research has also focused on seeds as a valuable oil source. From the pot marigold seeds, about 20% of oil can be extracted, in which, among the isomers of conjugated linolenic acids (CLNAs), α -calendic acid (8E, 10E, 12 Z-octadecatrienoic acid) is predominant and β -calendic acid (8E, 10E, 12 E) occurs in small amounts [16]. Oils containing conjugated trienoic acids have specific properties and this makes them useful in the production of high quality paints and polymers [3]. Conjugated linolenic acids have shown important health

benefits. It was recently proved that CLNAs are effective at inhibiting carcinogenesis including inhibition of proliferation [25, 26] and suppression of invasion and adhesion [22]. The studies have demonstrated that CLNAs could induce apoptosis in different human cancers such as colorectal adenocarcinoma [11], promyelocytic leukemia [10], mammary cancer and bladder cancer [20]. Furthermore, study by Li, Q. *et al.* [14] showed anticancer effects of α -calendic acid and β -calendic acid in choriocarcinoma JEG-3 cells. The results of studies have also indicated anti-obesity [1], antioxidant [18], and anti-inflammatory effects [13] of CLNAs and their abilities to decrease blood pressure [12]. Because CLNAs may be useful in the prevention and fight against many diseases, products with their high content are searched for. The usefulness of seeds of individual pot marigold cultivars for pharmaceutical industry depends on many factors. One of the most important factors is the content and composition of seed oil, which

depends on genetic characteristics [7] as well as environmental and climatic conditions [5].

The aim of the study was to determine the main factors that affect the content and composition of oil from seeds of ten pot marigold cultivars grown in Poland.

Materials and Methods

Plant material

The seeds were collected from 10 different cultivars of pot marigold ('Tokaj' - denoted as PM1, 'Santana' - PM2, 'Radio' - PM3, 'Persimmon Beauty' - PM4, 'Orange King' - PM5, 'Promyk' - PM 6, 'Szlem' - PM7, 'Mandarin Twist' - PM8, 'Apricot Twist' - PM9, 'Geisha Girl' - PM 10) grown on the experimental plot of the University of Life Sciences in Lublin during the 2010 growing season. In the third decade of August (time when about 50 - 60% of seeds reached maturity), the herbicide diquat was applied to desiccate the plants. The seeds were harvested 7 days later, then threshed, and cleaned.

Oil extraction and methyl-esterification

The seeds from the individual cultivars were ground separately using a stainless-steel grinder and fat was extracted from 5g samples during 8 h with n-hexane using a Soxhlet apparatus. In the next step, n-hexane was evaporated at 40 °C from the extract by means of a rotary evaporator and the resulting oil was stored in a freezer at a temperature of -18°C. Methyl-esterification of the lipids obtained was carried out using the BF₃-CH₃OH method according to AOCS [17].

GC analysis

For the analysis, a Varian 3800 (Walnut Creek, CA USA) gas chromatograph equipped with an auto

injector and a flame-ionizing detector (FID) was used. Cis-trans fatty acid methyl esters (FAMES) were separated by means of an UltiMetalTM capillary column CP-WAX 52CB (df 0.25 µm, length 60 m) with a polyethylene glycol stationary phase. Helium with a flow rate of 1.4 mL x min⁻¹ was used as a gas carrier. The initial temperature of the column was 120°C, the temperature increase was 2°C min⁻¹, and the maximum temperature was 210°C. The duration of the analysis was 127 min. The injector and detector temperatures were set to 160°C. FAMES peaks were identified based on the Supelco 37 Component FAME Mix and LGC standards. The reported results for the fatty acid composition are averages of triplicate measurements. The statistical analysis was carried out using the Statgraphics Centurion XVI software (v. 16.1.03).

Results and Discussion

The oil contents in the seeds of the evaluated cultivars of pot marigold ranged from 14.67% to 19.86% (Table I). Evidently, the largest amounts of oil were found in the samples from cultivars "Orange King" (19.76 ± 0.10%) and 'Tokaj' (19.33 ± 0.20%), and the lowest amounts in cultivars 'Szlem' (14.88 ± 0.26%) and 'Apricot Twist' (15.28 ± 0.11%). The oil contents were similar to those reported by Cromack and Smith [5] (14.20-19.20%, England), Martin *et al.* [15] (17.1-21.4%, New Zealand), Dulf *et al.* [7] (13.6-21.7%, Romania), and Gesch [8] (18.6-20.5%, USA). On the other hand, the oil contents reported by Angelini *et al.* [2] (2.0-12.0%, Italy) and Özgül-Yücel [16] (5.9%, Turkey) were much lower.

Table I
Oil contents (%) and saturated and monounsaturated fatty acids composition (%) of different cultivars of pot marigold seed oils (n = 3)

Cultivar	Oil content	Saturated acids			Monounsaturated acids	
		16:0 Palmitic	18:0 Stearic	Other ^b	18:1n9c Oleic	Other ^c
PM 1	19.33 ± 0.20 ^a	4.25 ± 0.19	3.40 ± 0.27	1.41 ± 0.17	5.14 ± 0.54	1.08 ± 0.21
PM 2	16.54 ± 0.09	5.27 ± 0.56	2.20 ± 0.22	1.52 ± 0.33	5.53 ± 0.50	1.29 ± 0.10
PM 3	17.27 ± 0.16	5.35 ± 0.21	2.41 ± 0.45	1.36 ± 0.33	4.63 ± 0.33	1.28 ± 0.29
PM 4	18.37 ± 0.11	4.32 ± 0.33	1.34 ± 0.17	0.88 ± 0.09	4.59 ± 0.11	1.32 ± 0.26
PM 5	19.76 ± 0.10	4.36 ± 0.15	2.45 ± 0.28	2.48 ± 0.21	5.50 ± 0.25	1.87 ± 0.26
PM 6	16.21 ± 0.14	4.75 ± 0.19	1.68 ± 0.12	2.86 ± 0.16	5.10 ± 0.20	1.15 ± 0.08
PM 7	14.88 ± 0.26	5.39 ± 0.27	2.29 ± 0.28	2.80 ± 0.21	4.73 ± 0.48	1.49 ± 0.24
PM 8	16.97 ± 0.14	3.58 ± 0.50	2.52 ± 0.23	1.56 ± 0.13	5.24 ± 0.28	0.79 ± 0.17
PM 9	15.28 ± 0.11	4.51 ± 0.37	4.51 ± 0.35	1.17 ± 0.10	5.78 ± 0.78	0.60 ± 0.09
PM 10	18.63 ± 0.25	3.43 ± 0.31	2.46 ± 0.31	0.72 ± 0.08	3.64 ± 0.54	0.75 ± 0.12

^a Mean ± standard deviation, ^b Σ 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 17:0, 20:0, 22:0, 24:0; ^c Σ 15:1, 16:1, 17:1, 18:1n7t, 20:1n9c, 20:1n7c

Based on the ranges of values obtained in individual studies, it can be concluded that the selection of a suitable genotype of pot marigold has a significant impact on the amount of extracted oil.

Moreover, it can be seen that the oil contents from the crops cultivated in the temperate climate [5, 7, 15] are greater than those obtained in the Mediterranean climate [2, 16].

Twenty six fatty acids were identified in the analysed cultivars. Saturated fatty acids (SFAs)

were in the range of 6.54 - 10.48% of the total amounts (Figure 1).

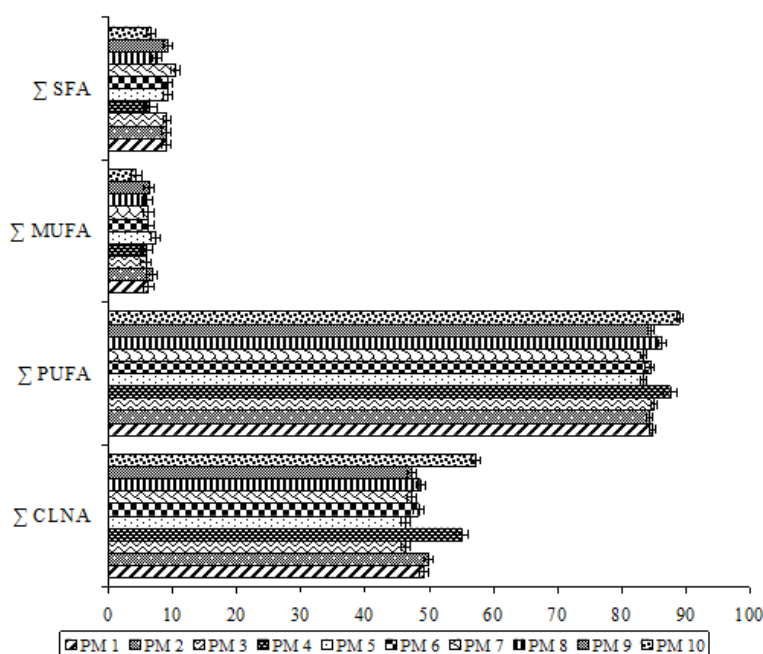


Figure 1.

Contents (%) of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and conjugated linoleic acids (CLNA) in pot marigold seed oils of different cultivars

The predominant were palmitic acid ($4.52 \pm 0.98\%$) and stearic acid ($2.43 \pm 1.09\%$) (Table I), the most common saturated fatty acids in animal and plant fats. The amounts of other saturated fatty acids were below 1%. The average content of SFAs obtained in our study was 8.63%, whereas Dulf *et al.* [7] obtained 6.99%, Martin *et al.* [15] 14.07% and Özgül-Yücel [16] 17.6%.

The contents of monounsaturated fatty acids (MUFAs) were in the range of 4.39 - 7.37% (Figure 1) and oleic acid ($4.99 \pm 1.07\%$) was the predominant

compound in this group (Table I). The calendula seed oils of the examined cultivars were abundant in polyunsaturated fatty acids (83.30 - 89.00%) (Figure 1). The most common among them were CLNAs (46.29 - 57.14%). In the group of polyunsaturated acids, α -calendic acid ($43.7 \pm 7.32\%$), linoleic acid ($34.21 \pm 2.96\%$), and β -calendic acid ($5.83 \pm 1.94\%$) were predominant (Table II). Other polyunsaturated fatty acids were found in the amounts below 1%.

Table II

Polyunsaturated fatty acid and conjugated linoleic acids content (%) of different cultivars of pot marigold seed oils (n = 3)

Cultivar	Polyunsaturated acids		Conjugated linoleic acids		Other ^h
	18:2n6 ^d Linoleic	18:3n3 ^e α -Linolenic	18:3n6 ^f α -Calendic	18:3n6 ^g β -Calendic	
PM 1	34.37 $\pm$ 0.45	0.83 $\pm$ 0.20	43.02 $\pm$ 1.49	6.15 $\pm$ 0.35	0.36 $\pm$ 0.04
PM 2	32.96 $\pm$ 0.80	0.90 $\pm$ 0.02	43.96 $\pm$ 1.26	5.98 $\pm$ 0.47	0.39 $\pm$ 0.03
PM 3	36.63 $\pm$ 0.54	1.77 $\pm$ 0.20	38.79 $\pm$ 0.69	7.50 $\pm$ 0.32	0.29 $\pm$ 0.04
PM 4	31.11 $\pm$ 0.35	0.93 $\pm$ 0.09	51.46 $\pm$ 0.22	3.65 $\pm$ 0.41	0.39 $\pm$ 0.06
PM 5	35.23 $\pm$ 0.51	1.54 $\pm$ 0.28	40.86 $\pm$ 0.77	5.43 $\pm$ 0.50	0.28 $\pm$ 0.04
PM 6	34.52 $\pm$ 0.40	1.08 $\pm$ 0.11	43.12 $\pm$ 0.48	5.28 $\pm$ 0.17	0.47 $\pm$ 0.03
PM 7	34.42 $\pm$ 0.53	1.21 $\pm$ 0.12	40.65 $\pm$ 0.42	6.51 $\pm$ 0.39	0.51 $\pm$ 0.02
PM 8	36.47 $\pm$ 0.33	0.84 $\pm$ 0.01	41.06 $\pm$ 0.74	7.53 $\pm$ 0.41	0.41 $\pm$ 0.06
PM 9	35.73 $\pm$ 0.56	1.08 $\pm$ 0.06	40.63 $\pm$ 0.45	6.58 $\pm$ 0.46	0.40 $\pm$ 0.01
PM 10	30.70 $\pm$ 0.52	0.87 $\pm$ 0.07	53.43 $\pm$ 0.57	3.71 $\pm$ 0.28	0.28 $\pm$ 0.04

^d(9c,12c), ^e(9c,12c,15c), ^f(8t,10t,12c), ^g(8t,10t,12t); ^h Σ 20:2n6, 20:3n3, 20:4n6

The average content of α -calendic acid, the dominant component of CLNAs in the pot marigold seed oil, was 43.70 % in our study, whereas Dulf *et al.* [7]

obtained 54.66%, Cromack and Smith [5] 46.77%, Štolcová *et al.* [19] 45.09%, Angelini *et al.* [2] 36.73%, Martin *et al.* [15] 35.86%, and Özgül-Yücel [16] 17.6%.

The statistical analysis indicated that the above contents of α -calendic acid were significantly positively correlated with the average monthly precipitation in the warmest month of the year (July in the northern and January in the southern hemisphere) of particular crop location ($r = 0.845$, $p = 0.017$; 79.0 mm for Zamosc (this study), 81.0 mm for Cluj-Napoca [7], 66.2 mm for Prague [19], 45.7mm for Christchurch [15], 55 mm for Plymouth [5], 24.0 mm for Pisa [2], and 19.1 mm for Istanbul [16] (the climate data were taken from [23])). They were significantly inversely correlated at 90% probability level with the average monthly temperatures ($r = -0.717$, $p = 0.070$; 16.1, 17.1, 17.4, 17.4, 18.2, 22.9, and 23.2°C for Plymouth, Prague, Christchurch, Zamosc, Cluj-Napoca, Pisa, and Istanbul respectively [23])). Thus, an increase in temperature in the period of seed maturation decreased the contents of α -calendic acid and other unsaturated fatty acids. The same relationship between the content of α -calendic acid and temperature was indicated previously in a laboratory study by Cahoon *et al.* [4]. The authors determined a small amount or no acid in the

cultures of transgenic soybean embryos at 30°C, whereas the growth was enhanced significantly at lower temperatures. Of course the statistical analysis carried out in such a way ignores many genetic and practical aspects, e.g. the fact that in the above locations different cultivars were used, whose selection and cultivation have been optimized for climate conditions. However, in the authors' opinion, this does not discredit the statement but confirms that climatic conditions are one of the major factors influencing the composition of fatty acids in pot marigold seeds. The relationship between the content of α -calendic acid and climatic conditions has not been reported in the literature to date.

Another very important factor that influences both CLNAs and the composition of other fatty acids in pot marigold oil is the genotype of the crop. This can be seen in Table II in our study and also in the studies of Dulf *et al.* [7], Cromack and Smith [5], Martin *et al.* [15], or Angelini *et al.* [2].

Table III shows values of Pearson's correlation coefficients and partial correlation coefficients for the main fatty acids of pot marigold oil.

Table III

Values of Pearson's correlation coefficients (left side), partial correlations (right side; in any case, the effect of four other variables was taken into account) and their p-values (in subscript brackets) for the main fatty acids of the pot marigold oils ($n = 30$)

	Palmitic	Stearic	Oleic	Linoleic	α -Calendic	β -Calendic
Palmitic		-0.275 _(0.174)	-0.591 _(0.002)	-0.658 _(<0.001)	-0.776 _(<0.001)	-0.135 _(0.510)
Stearic	-0.084 _(0.661)		-0.164 _(0.425)	-0.051 _(0.806)	-0.125 _(0.542)	0.157 _(0.445)
Oleic	0.089 _(0.641)	0.131 _(0.491)		-0.566 _(0.003)	-0.716 _(<0.001)	-0.164 _(0.422)
Linoleic	0.284 _(0.129)	0.447 _(0.013)	0.422 _(0.020)		-0.860 _(<0.001)	0.085 _(0.680)
α -Calendic	-0.484 _(0.007)	-0.387 _(0.035)	-0.558 _(0.001)	-0.936 _(<0.001)		-0.345 _(0.085)
β -Calendic	0.326 _(0.079)	0.459 _(0.011)	0.414 _(0.023)	0.883 _(<0.001)	-0.887 _(<0.001)	

As can be seen, the values of those coefficients are not consistent in some cases. During interpretation of the correlations between fatty acids, one should take into account that certain acids were formed from others, resulting in negative correlations. Moreover, the fact that the sum of fatty acids in oil is equal to 100% causes collinearity problems. In such a case, when both Pearson's and partial correlations are significant and indicate the same positive or negative relationship, this result cannot be questioned. On the other hand, when Pearson's and partial correlations are not consistent, partial correlation values are usually more correct. However, in some cases, also partial correlations may give false results [24]. Both Pearson's and the partial correlation coefficients indicated a very strong negative relationship between linoleic and α -calendic acid. This correlation results from the fact that α -calendic acid is synthesized from linoleic acid (modification of the Δ^9 double bond by FAD2 related enzymes [4]). The correlation coefficients in Table III indicated a significant negative relation-

ship also between the oleic and the α -calendic acids ($r = -0.558$, $p = 0.001$ – Pearson; $r = -0.716$, $p < 0.001$ – partial correlation). According to Crombie and Holloway [6], oleic acid is the second precursor of α -calendic acid. In the case of β -calendic acid, the only significant partial correlation was the negative one with α -calendic acid. The significant negative relationship between the α - and β -calendic acids (Table III) suggests that β -calendic acid is synthesized from α -calendic acid. To the best of the author's knowledge, this is the first report on the statistical relationship between the α - and β -calendic acid. However, the above isomerization reaction of a double bond at Δ^{12} position of the acid was suggested previously by Takagi and Itabashi [21].

The content of β -calendic acid in our study ($5.83\% \pm 1.94\%$) (Table II) was larger than that obtained by Dulf *et al.* [7] ($0.69\% \pm 0.23\%$) but much lower than the value reported by Özgül-Yücel [16] (11.2%). It can be supposed that the differences largely resulted from climate conditions (mainly

temperature), which affected the enzymatic activity during seed maturation.

Conclusions

The seed oils of the ten cultivars of pot marigold grown in Poland were rich in CLNAs (from 46.29% to 57.14% of total fatty acids). It was indicated that in addition to the effect of cultivars, the determined large amounts of CLNAs resulted from a relatively low average temperature and high precipitation rates during seed maturation. The statistical analysis suggested that linoleic acid was the main precursor of α -calendic acid, and α -calendic acid was a precursor of β -calendic acid. The comparison of the results obtained with those shown by other authors clearly indicates that both the oil content in seeds and the content of α -calendic acid in the oil will be the largest in crops cultivated in colder climate with high precipitation. In turn, lower contents of oil and larger contents of β -calendic acid can be expected in crops grown in warmer, dry climate.

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