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RESEARCH PAPER

Determination of free apocarotenoids and apocarotenoid esters in human colostrum

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Abstract

The presence of carotenoids in human colostrum has been reported in the literature, and xanthophyll esters in human colostrum were recently detected for the first time. However, no published studies have reported whether apocarotenoids, which are metabolites derived from carotenoid enzymatic or nonenzymatic oxidative cleavage, are present in human colostrum. Therefore, the purpose of the present study was to search for the possible occurrence of apocarotenoids, including apocarotenoid esters, in human colostrum for the first time by applying an online supercritical fluid extraction–supercritical fluid chromatography–tandem mass spectrometry methodology. Recent evidence related to apocarotenoid transcriptional activity has suggested that they may have beneficial health properties superior to those of their parent carotenoids. Three different apocarotenoids, namely apo-8′-β-carotenal, apo-8′-lycopenal, and β-citraurin, were identified in intact human colostrum samples, with average concentrations of 85 nmol L^{-1}, 54.6 nmol L^{-1}, and 75.4 nmol L^{-1}, respectively. The overall detection of 16 different free apocarotenoids and 10 different apocarotenoid fatty acid esters in human colostrum was achieved here for the first time. Their occurrence in human colostrum certainly has implications for newborn health status, since colostrum is the only form of food for the newborn during the very first days of life.

Keywords Apocarotenoids · Carotenoids · Human colostrum · SFE-SFC-APCI/QqQ/MS

Abbreviations

APCI	Atmospheric pressure chemical ionization
MS	Mass spectrometry
QqQ	Triple quadrupole
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction

Introduction

The role of apocarotenoids, which are metabolites derived from carotenoid enzymatic or nonenzymatic oxidative cleavage, is emerging in mammals [1, 2]. The physiological and biological activity of apocarotenoids has been reviewed and

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their role in gene expression and modulation of ligand-activated nuclear receptors has been outlined [3, 4], suggesting that they are involved in different biological activity such as the possible prevention of some types of cancer, In fact, probably due to their transcriptional activity, apocarotenoids may have beneficial health properties superior to those of their parent carotenoids.

Apocarotenoids may be absorbed from the diet or may be produced by the parent carotenoid cleavage enzymes. Enzymatic cleavage is known to occur in humans either by central carotenoid cleavage catalyzed by the β-carotene-15,15′-oxygenase (BCO1) or by eccentric cleavage catalyzed by the β-carotene-9,10′-oxygenase (BCO2); other eccentric cleavage may take place by nonenzymatic or enzymatic processes [1]. Figure 1 shows an example of possible eccentric oxidative cleavage sites of zeaxanthin leading to the production of various apozeaxanthinals, apo-8′-zeaxanthinal (β-citraurin) for example being widely distributed in *Citrus* species [6]. Apocarotenoids in plants are known to be produced by nonenzymatic oxidation or by different carotenoid cleavage dioxygenase enzymes (CCDs) [7]. Researchers have recently reported the detection of apocarotenoids in intact human blood by online supercritical fluid extraction–supercritical fluid chromatography–tandem mass spectrometry (SFE-SFC-QqQ/MS) [8]. The presence of carotenoids in human colostrum has been reported in various studies [9–13], and xanthophyll esters in human colostrum were recently detected for the first time by Rios et al. [14]; carotenoid esterification with fatty acids provides greater stability to the molecules, may alter their physical properties, and specifically modifies the carotenoid profile in human colostrum. The same significance can probably be attributed to apocarotenoid esterification with fatty acids.

No reports are available on the presence of apocarotenoids in human colostrum. Therefore, the purpose of the present study was to search for the possible occurrence of apocarotenoids, including apocarotenoid esters, in human colostrum for the first time by applying an online SFE-SFC-MS methodology.

Materials and methods

Chemicals

Reagents were purchased from Merck Life Sciences (Merck KGaA, Darmstadt, Germany). Carotenoid standards capsanthin, β-carotene, β-cryptoxanthin, and zeaxanthin were obtained from Extrasynthese (Genay, France), and apocarotenoid standards apo-8′-β-carotenal, apo-8′-lycopenal, and β-citraurin (apo-8′-zeaxanthinal) were acquired from CaroteNature GmbH (Münsingen, Switzerland). For method validation, colostrum samples were used. Carotenoid and apocarotenoid stock solutions were prepared using a 1:1 (*v*/*v*) mixture of hexane and CH_2Cl_2 at a concentration of 1000 mg L^{-1}, and the solutions were stored in vials in the dark at −20 °C. Calibration standard solutions were prepared in colostrum (matrix-matched calibration).

In addition, a series of β-apocarotenals, apolycopenals, apocanthaxanthinals, apozeaxanthinals, apocapsorubinals, and ε-apoluteinals were generated by oxidative cleavage of β-carotene, lycopene, canthaxanthin, zeaxanthin, capsorubin, and lutein, respectively, following the procedure described by Rodriguez and Rodriguez-Amaya for β-carotene [15], and also reported by Giuffrida et al. for zeaxanthin and capsorubin [16], and Zoccali et al. for lutein [8]. The lycopene and canthaxanthin standards used for the oxidative cleavage were kindly donated by colleagues.

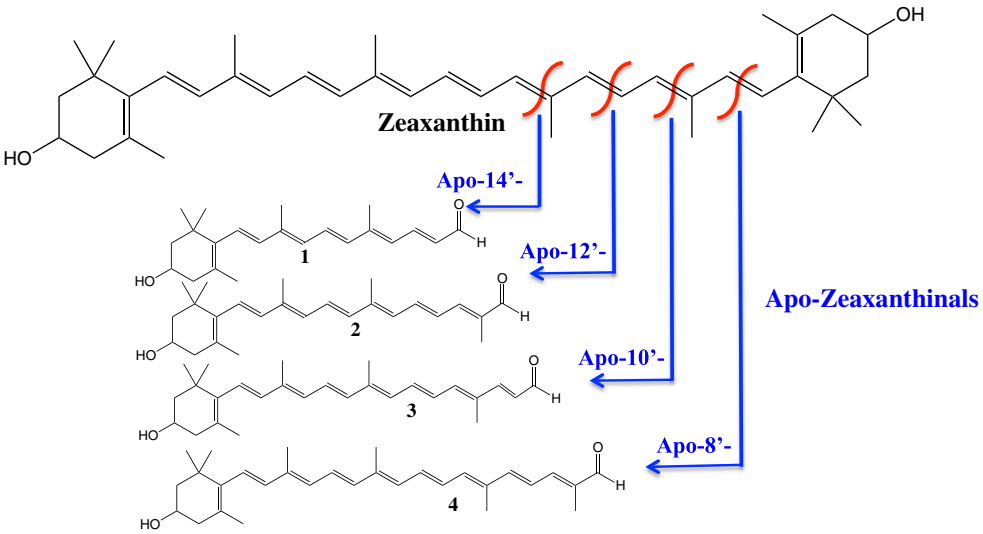

Fig. 1 Possible eccentric oxidative cleavage sites of zeaxanthin leading to the production of various apozeaxanthinals. (**1**) Apo-14′-zeaxanthinal. (**2**) Apo-12′-zeaxanthinal. (**3**) Apo-10′-zeaxanthinal. (**4**) Apo-8′-zeaxanthinal. Reprinted with permission from Ref. [5]. Copyright 2018 ACS

Sample collection

Aliquots of a few milliliters of colostrum were collected from six women volunteers, who had a normal diet without any type of carotenoid supplementation; the samples were collected from the volunteers at the third or fifth postpartum day.

SFE-SFC-APCI-MS instrumentation

A Shimadzu Nexera-UC system (Shimadzu, Japan) was used for the analyses; the characteristics of the instrument were described in Zoccali et al. [8].

SFE-SFC-APCI-MS analyses

The analytical conditions used for the analyses were modified slightly from the report by Zoccali et al. [8] in order to optimize the chromatographic separation for the analytes investigated. Twenty-microliter aliquots of intact colostrum samples were utilized for each analysis, with no sample preparation step. Solvent (A) CO_2 and solvent (B) CH_3OH were used for the SFE as follows: from 0 to 3 min, 20% CH_3OH, from 3 to 5 min, 0% B. Flow rate: 2 mL min^{-1}; extraction mode: 0–3 min static mode, 3–5 dynamic mode; back-pressure regulator: 150 bar; extraction vessel temperature: 40 °C. Solvent (A) CO_2 and solvent (B) CH_3OH were used for the SFC as follows: from 5 to 22 min increasing from 0 to 80% B, then from 22 to 23 min increasing to 100% B, and then 100% B for 2 min. Flow rate: 2 mL min^{-1}. Make-up solvent CH_3OH at flow 1.2 mL min^{-1}. Chromatography was carried out on an Ascentis Express C30, 150 mm × 4.6 mm × 2.7 μm d.p. (Merck KGaA). The column oven temperature was 40 °C and the-back pressure regulator was 150 bar. Standard injection volume was 2 μL. The mass spectrometer conditions were as reported in Zoccali et al. [8]. Briefly, a Shimadzu (Japan) LCMS-8050 triple-quadrupole mass spectrometer equipped with an APCI source was used, and the MS conditions were set as follows: acquisition mode: SCAN (±), (selective ion monitoring) SIM (−), and (multiple reaction monitoring) MRM. Interface temperature: 350 °C; DL temperature: 200 °C; block heater temperature: 200 °C; nebulizing gas flow (N_2) 3 L min^{-1}; drying gas flow (N_2) 5 L min^{-1}; full scan range: 200–1200 *m/z*; event time 0.05 s for each event. Carotenoids and apocarotenoids were identified using the available standard, full scan, and selected ion monitoring (SIM). Multiple reaction monitoring (MRM) experiments were also carried out for the identification of carotenoids and apocarotenoids according to previously optimized transitions reported for carotenoids, β-apocarotenals, apozeaxanthinals, apocapsorubinals, and ε-apoluteinals [8, 16]. MRM experiments were carried out to optimize the transitions for the identification of apolycopenals and apocanthaxanthinals in this study; the qualifier and quantifier ions were selected by carrying out product ion scan experiments at different collision energies in both positive and negative APCI ionization modes.

Method validation

Mixtures of carotenoid and apocarotenoid standards at concentrations of 5000, 10,000, and 50,000 μg L^{-1} in hexane were used with spiking concentrations of 500, 1000, and 5000 μg L^{-1} for recovery calculations.

Recovery and repeatability (expressed as %CV) experiments, performed on the colostrum sample of a volunteer, were carried out at three concentrations of 500, 1000, and 5000 μg L^{-1}) ($n = 5$). The colostrum sample was analyzed previously in order to subtract the detected area of the targeted carotenoids and apocarotenoids.

Matrix-matched linearity was tested by performing four replicates at each concentration level. A total of five levels were selected, ranging from 50 to 5000 μg L^{-1} (50, 100, 500, 1000, and 5000 μg L^{-1}).

The least-squares method was used to estimate the regression line for the construction of the calibration curves. The linearity and goodness-of-fit measurements of the curves were confirmed using Mandel's fitting tests.

For the calculation of both the limit of quantification (LoQ) and limit of detection (LoD), the standard deviation of the analyte area relative to the colostrum sample fortified at the lowest concentration level ($n = 4$) was multiplied three and ten times, respectively, and the result was divided by the slope of the calibration curve.

The significance of the intercept was established by means of a *t* test. All the statistical tests were carried out at the 5% significance level. For absolute quantification purposes, matrix-matched calibration was used.

Results and discussion

Methodology

The method employed in the present work was developed by Zoccali et al. [8] and is only slightly modified here in order to improve the chromatographic separation for the analyzed compounds as described above. A detailed scheme and operation mode for the SFE-SFC-MS system were reported by Zoccali et al. [17, 18]. Briefly, the system operates in three steps: (1) SFE static extraction mode, (2) SFE dynamic extraction mode, and (3) SFC analysis. The main objective of the present study was the extraction and detection of targeted carotenoids and apocarotenoids from colostrum samples using supercritical fluid and tandem mass spectrometry detection. The method has also been applied for the quantification of carotenoids and apocarotenoids belonging to different classes

using the available standards, namely capsanthin (β,k-rings, hydroxyl- and carbonyl functions), β-carotene (hydrocarbon), β-cryptoxanthin (monohydroxy-), zeaxanthin (dihydroxy-), apo-8′-β-carotenal (8′-apo-β-caroten-8′-al), apo-8′-lycopenal (8′-apo-ψ-caroten-8′-al), and β-citraurin [(3R)-3-hydroxy-8′-apo-β-caroten-8′-al].

The following parameters were considered for method validation: LoQ and LoD, linearity, recovery, and repeatability. The LoQ was in the range of 15–30 μg L^{-1} range, and the LoD in the range of 4–9 μg L^{-1}, as shown in Table 1.

Three levels, namely 500, 1000, and 5000 μg L^{-1} ($n = 5$), were used for the calculation of recovery and repeatability; the recoveries were in the range of 84–102% (500 μg L^{-1}), 89–119% (1000 μg L^{-1}), and 81–120% (5000 μg L^{-1}), respectively. These experiments were carried out on colostrum samples.

The calculated values are also reported in Table 1. The proper method linearity was not calculated, but the method applied was linear in the range of LoQ to 5000 μg L^{-1} for all compounds. The linear range was measured using spiked colostrum samples and evaluated using Mandel's fitting test (Fcalc < Ftab) in the range of 50–5000 μg L^{-1}.

SFE-SFC-MS intact colostrum analyses

Previously reported carotenoid determination in human colostrum was carried out by means of separate liquid extraction and liquid chromatography approaches [9–14]. An online SFE-SFC-MS methodology was applied here for the first time for both the identification and quantification of selected carotenoids and apocarotenoids in human colostrum. The online nature of this technique provides high sensitivity, minimizing sample losses. The colostrum samples were analyzed without any sample preparation or carotenoid ester saponification steps.

Four selected carotenoids and three selected apocarotenoids having different functional moieties were identified and quantified in samples of human colostrum as shown in Table 2. The applied methodology allowed for the detection of 16 different free apocarotenoids and 10 different apocarotenoid fatty acid esters in human colostrum for the first time, as shown in Table 3 together with the relative MRM experimental data. Table 3 also reports the optimized transitions for the detected apolycopenals and apocanthaxanthinals. The quantified isobaric apo-8′-β-carotenal and apo-8′-lycopenal were clearly identified by their different chromatographic retention times and the characteristic qualifier ion at *m/z* 69 for the apo-8′-lycopenal, produced by bis-allylic cleavage of the single bond adjacent to the chromophore of the acyclic end group, which was not produced in the case of the apo-8′-β-carotenal. The other isobaric apo-β-carotenals and apo-lycopenals were identified by the different optimized transitions and Q/q ratios; they all produced quantifier and qualifier ions with values below *m/z* 210 and with fragment ions 14 amu apart, corresponding to fragmentation of the polyene chain and possible transfer of a hydrogen atom to a neutral fragment, in agreement with the report by van Breemen et al. [19] for carotenoids containing aldehyde or acyclic terminus. Interestingly, the apo-10′-, apo-12′-, and apo-14′- lycopenals bearing a shorter polyenic chain did not produce the ion at *m/z* 69 as either the qualifier or the quantifier. The two identified 4-oxo-β-apo-carotenals, apo-10′- and apo-12′-canthaxanthinals, produced ions at values below *m/z* 210, likely because the electron-withdrawing carbonyl group increased the probability of fragmentation within the polyene system by reducing the π-electron density of the carbon skeleton, as was reported for canthaxanthin by van Breemen et al. [19]. A series of 4-oxo-β-apo-carotenals were reported to be generated upon oxidation on canthaxanthin by oxygen bubbling in a standard solution [20]. Also, interestingly, only ε-

Table 1 Validation parameter values determined for the selected carotenoids and apocarotenoids analyzed by the SFE-SFC-QqQ/MS method in colostrum samples

	Zeaxanthin	Capsanthin	β-Carotene	β-Cryptoxanthin	Apo-8′-lycopenal	β-Citraurin	β-Apo-8'-carotenal
LoQ (μg L^{-1})	20	16	17	30	18	20	15
LoD (μg L^{-1})	6	5	5	9	5	6	4
LoQ (nmol L^{-1})	35.2	27.4	31.7	54.3	43.3	46.3	36.1
LoD (nmol L^{-1})	10.6	8.6	9.3	16.3	12.0	13.9	9.6
Recovery % (500 μg L^{-1})	92	91	85	84	87	102	91
Repeatability CV%	9	6	15	7	8	12	19
Recovery % (1000 μg L^{-1})	115	112	89	90	110	119	109
Repeatability CV%	12	10	14	8	9	13	14
Recovery % (5000 μg L^{-1})	103	99	108	93	81	114	120
Repeatability CV%	17	15	17	16	7	15	18
Linearity (μg L^{-1})	50–5000	50–5000	50–5000	50–5000	50–5000	50–5000	50–5000

Table 2 Carotenoid and apocarotenoid quantification in six different colostrum samples by SFE-SFC-QqQ/MS

Volunteer	Zeaxanthin		Capsanthin		β-Carotene		β-Cryptoxanthin		Apo-8′-lycopenal		β-Citraurin		β-Apo-8'-carotenal	
	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}	μg dL^{-1}	nmol L^{-1}
1	60.6	1066.2	29.5	505.0	27.0	504.1	48.2	872.8	2.9	70.5	4.5	104.4	3.3	79.2
2	< LoD	< LoD	24.8	425.1	19.5	364.2	47.2	855.1	2.3	55.4	3.9	90.2	3.3	78.1
3	59.8	1052.9	24.1	412.5	72.4	1350.1	56.1	1016.9	2.1	49.6	3.1	73.0	4.9	119.0
4	59.5	1047.4	23.9	408.6	21.2	396.1	51.9	941.0	2.5	60.6	2.7	58.7	3.0	72.6
5	58.9	1037.6	23.5	407.7	< LoD	< LoD	49.8	901.9	1.9	46.5	2.5	51.8	4.7	113.0
6	58.4	1028.9	23.5	407.2	< LoD	< LoD	47.5	860.9	1.9	44.7	3.2	74.4	2.0	48.1
Min	< LoD	< LoD	23.5	407.2	< LoD	< LoD	47.2	855.2	1.9	44.7	2.5	51.8	2.0	48.1
Max	60.6	1066.2	29.5	505.0	72.4	1350.1	56.1	1016.9	2.9	70.5	4.5	104.4	4.9	119
Average	49.5	872.2	24.9	427.7	23.4	435.8	50.1	908.1	2.3	54.6	3.3	75.4	3.5	85.0
± SD	22.2	390.2	2.1	35.1	24.2	452.2	3.1	56.6	0.4	8.9	0.7	17.8	1.0	24.3

SD = (±) standard deviation

LoD = limit of detection

Table 3 Overall apocarotenoids and apocarotenoid esters detected by SFE-SFC-APCI(+/−)/QqQ MS analysis in colostrum samples and MRM experimental data determined in positive APCI ion mode

Compound	Quantifier	CE	Qualifier	CE	Ion Q/q ratio % (+)	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6
β-citraurin	433 > 105	−45	433 > 119	−35	85	×	×	×	×	×	×
Apo-12′-zeaxanthinal	367 > 105	−35	367 > 119	−30	80	×	×	×	×	×	×
β-Apo-8'-carotenal	417 > 119	−30	417 > 105	−35	73	×	×	×	×	×	×
β-Apo-10'-carotenal	377 > 105	−35	377 > 119	−30	83	×		×	×	×	
β-Apo-12'-carotenal	351 > 105	−35	351 > 119	−25	74	×	×			×	
β-Apo-14'-carotenal	311 > 105	−25	311 > 119	−25	77		×		×		
Apo-8′-lycopenal	417 > 105	−40	417 > 69	−25	89	×	×	×	×	×	×
Apo-10′-lycopenal	377 > 91	−50	377 > 105	−50	58	×	×	×	×	×	×
Apo-12′-lycopenal	351 > 95	−30	351 > 77	−50	68		×		×	×	×
Apo-14′-lycopenal	311 > 91	−40	311 > 77	−50	92	×	×	×	×	×	×
ε-Apo-8-luteinal	415 > 119	−40	415 > 91	−50	95	×	×	×	×	×	×
Apo-10'-capsorubinal	409 > 109	−20	409 > 127	−15	11	×	×	×	×	×	×
Apo-12'-capsorubinal	383 > 109	−20	383 > 127	−15	10	×	×	×	×	×	×
Apo-14'-capsorubinal	342 > 109	−20	342 > 127	−15	13			×	×	×	×
Apo-10'-canthaxanthinal	381 > 133	−24	381 > 203	−13	52	×	×	×	×	×	×
Apo-12'-canthaxanthinal	365 > 133	−23	365 > 105	−43	10	×	×	×	×	×	
Apo-8'-capsorubinal-C12:0	631 > 109	−30	631 > 127	−20	13	×	×		×		
Apo-8'-capsorubinal-C16:0	687 > 109	−35	687 > 127	−20	10				×		
Apo-10′-zeaxanthinal-C4:0	463 > 105	−40	463 > 119	−35	78		×	×	×		×
Apo-10′-zeaxanthinal-C10:0	547 > 105	−35	547 > 119	−30	87	×	×	×	×	×	×
Apo-10′-zeaxanthinal-C12:0	575 > 119	−30	575 > 105	−35	75	×	×	×	×	×	×
Apo-10′-zeaxanthinal-C14:0	603 > 105	−40	603 > 119	−30	77	×	×	×	×	×	×
Apo-8′-zeaxanthinal-C6:0	531 > 119	−40	531 > 105	−40	78		×				×
Apo-8′-zeaxanthinal-C8:0	559 > 119	−40	559 > 105	−40	78	×	×				
Apo-8′-zeaxanthinal-C10:0	587 > 119	−40	587 > 105	−40	81	×	×	×	×	×	×
Apo-8′-zeaxanthinal-C12:0	615 > 119	−40	615 > 105	−40	89		×	×	×	×	×

CE = collision energy (V)

apo-8-luteinal and only apo-12′-zeaxanthinal were detected among the different ε-apo-luteinals and apozeaxanthinals sought. Free capsanthin was also detected in human colostrum here for the first time; these reported qualitative findings may be related to the diet of the lactating mothers. The detected apocarotenoid esters were mainly esters of apo-10′- and apo-8′- zeaxanthinal. Fig. 2 shows the different MRM analysis enlargements of the carotenoids and apocarotenoids quantified in the human colostrum samples; all the analytes were analyzed in less than 18 min including the extraction step. The quantitative data reported in Table 2 for the carotenoids are within the range reported by Patton et al. [13], and compared with the values provided by Rios et al. [14], are higher for zeaxanthin, lower for β-carotene, and similar for β-cryptoxanthin. It has been reported that carotenoid levels are highest in colostrum, and that their content then decreases dramatically in mature milk [11–13], where no carotenoid esters were detected [14]. The present work represents the first report on both the identification and quantification of three different apocarotenoids, namely apo-8′-β-carotenal, apo-8′-lycopenal, and β-citraurin, in intact human colostrum; their average concentrations were 85 nmol L^{-1}, 54.6 nmol L^{-1}, and 75.4 nmol L^{-1}, respectively. Interestingly, the amounts of apo-8′-β-carotenal and β-citraurin reported here were around 14.9% and 6.6% of the corresponding main parent carotenoids β-carotene and zeaxanthin, respectively, although it should be taken into account that β-citraurin could be formed from oxidative cleavage of β-cryptoxanthin as well. The percentage of the quantified apo-8′-lycopenal relative to the parent lycopene was not calculated in this study, as the lycopene standard was kindly provided to us, and it was all used for the oxidative cleavage, as indicated in the Materials and methods section. Very few reports available in the literature provide data on the proportions of apocarotenoids relative to the corresponding main parent carotenoid in food and human fluids [21, 22]. Although the maternal diet certainly influences the carotenoid and apocarotenoid content in mammalian tissue, and the quantified amounts of apocarotenoids reported here are average values calculated from only six volunteers, they do provide a first indication of the order of magnitude at which apocarotenoids might be present in human colostrum. Moreover, the detection of 16 different free apocarotenoids and 10 different apocarotenoid fatty acid esters in human colostrum for the first time represents interesting evidence for

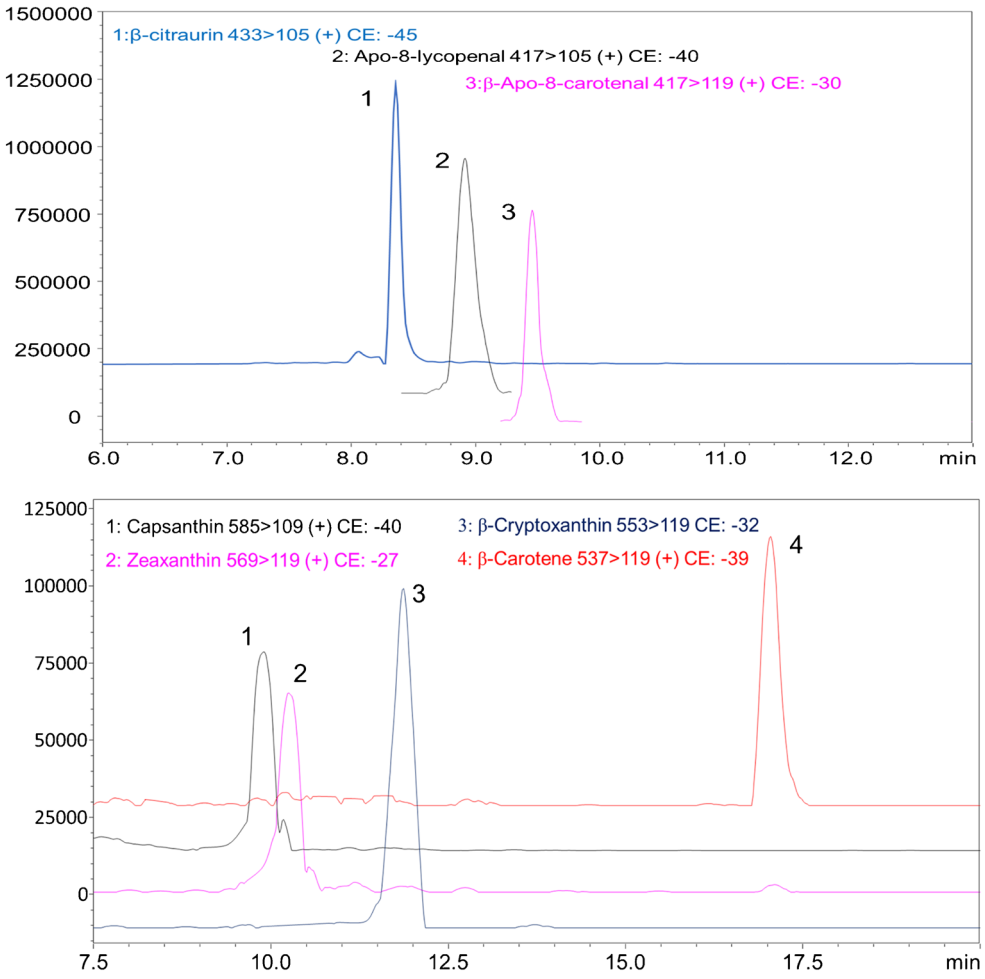

Fig. 2 MRM analysis enlargements of the different carotenoids and apocarotenoids quantified by SFE-SFC-APCI/QqQ/MS analysis in human colostrum samples

the possible role of these metabolites in mammalian colostrum, and consequently probably for the newborn; their occurrence in human colostrum certainly has implications for newborn health status, since colostrum is the only form of food for the newborn during the very first days of life.

Further studies will be needed to achieve a broader understanding of the occurrence of apocarotenoids in lactating mothers in different geographical areas. These findings thus further confirm the importance of these metabolites in mammals and encourage additional investigation of the possible presence and roles of the apocarotenoids in human biological fluids and tissues. Similar to the well-known occurrence of retinoids (retinaldehyde and its reduced product retinol or its oxidized product retinoic acid) in mammalian tissue [1–4], studies should also explore the possible occurrence of various non-retinoid apocarotenoid derivatives in different oxidative stages in human tissues and fluids generated by the reduction or oxidation of the corresponding aldehydic forms. In situ tissue-specific production of apocarotenoids has been suggested [23], although so far only the occurrence of apo-10'-lycopenol (the reduction product of apo-10'-lycopenal) has been detected in vivo in ferret lungs [24, 25]. In recent years, the occurrence and importance of long-chain acyl esters of carotenoids and apocarotenoids in food has also received increased attention [16, 26]. Therefore, the first report here of the detection of apocarotenoid esters in human colostrum further confirms the significance of the esterification process for carotenoid metabolites in human fluids as well.

Conclusions

The presence of non-retinoid apocarotenoids in human colostrum is reported here for the first time. The formation of free apocarotenoids may be related to their potential contribution to the regulation of various cellular functions and carotenoid homeostasis. The occurrence of the esterified forms is probably related to a more stable storage condition, likely similar to the vitamin A storage as retinyl esters in human tissue, although here the long-chain fatty acids are esterified with the secondary hydroxyl group of the β-ring of the detected apozeaxanthinal esters and the secondary hydroxyl group of the κ-ring of the detected apocapsorubinal ester moieties, and not at the primary hydroxyl group as occurs in the fatty acid esterification of retinol to form retinyl esters. Indeed, a very active acylation pathway in the mammary glands has been reported [14]. By using the described MRM approach, selected carotenoids and apocarotenoids were quantified directly, and an overall detection of 16 different free apocarotenoids and 10 different apocarotenoid fatty acid esters in human colostrum is reported for the first time. The system applied here can be regarded as a fast, sensitive, and reliable tool for the qualitative/quantitative determination of carotenoids and apocarotenoids in other human biological fluids and tissues, and could also be useful in large clinical surveys related to general oxidative health status and to enable a better understanding of the roles of these metabolites in human tissues.

Further studies will be undertaken to evaluate the possible occurrence of non-retinoid apocarotenoids in more advanced stages of lactation and in mature human milk.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study was carried out in compliance with ethical standards.

Sample collection was carried out at the Messina University Polyclinic Hospital, and although all participants were volunteers, they all provided written consent.

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