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Effects of turmeric powder on intestinal and biliary functions: The influence of curcuminoids concentration on spontaneous contractility

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ABSTRACT

Two turmeric food powders (C1 and C2) were studied for curcuminoid content and their effects on the guinea pig intestinal tract in vitro. C1 contained a higher curcuminoid content than C2 (5.22% vs 2.31%). C1 and C2 increased gallbladder (~10%) and biliary smooth muscle tone (~15%), without affecting the sphincter of Oddi smooth muscle contractility. C2 was more effective than C1 in lowering ileum tone (–22% vs –37%), whereas the reverse occurred in the colon (-50% vs –20%). Standard Fast Fourier transforms and absolute powers analysis of the frequency bands highlighted that, in the bile duct, C2 induced contractions of higher variability and ampler oscillations of low-frequency waves. At the Oddi sphincter, C1 had a biphasic effect, increasing and then drastically decreasing the oscillations. The same occurred with C2 in the ileum, while both samples reduced the fluctuations in the colon.

1. Introduction

Curcuma, Saffron of the Indies, or turmeric, native to India, is widely cultivated in regions with temperatures between 20 °C and 35 °C and high rainfall (Priyadarsini, 2014). Specifically, the rhizomatous root, collected at the end of the vegetative state, represents the part used to produce the drug. After cleaning and clearing from the rootlets, boiling for a few hours, and drying in the sun or in large ovens, the rhizomes are then crushed to obtain a yellow-orange powder. The rhizome contains nutritional components such as carbohydrates, proteins, fats, water, vitamins, and minerals. The phytochemical composition of turmeric includes numerous chemical classes, such as, among others, oxygenated sesquiterpenes and monoterpenes. The most represented metabolites are diarylheptanoids, of which curcuminoids, such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, are the most studied (S. Li, 2011; Serpa Guerra et al., 2020). Curcuminoids are phytochemicals of high biological interest, and the most important are curcumin (CU), demethoxyicurcumin (DMC), and bisdemethoxycurcumin (BDMC). The powder of Curcuma is used for both culinary and medical purposes (Serpa Guerra et al., 2020). Serpa Guerra et al. (2020) reported that there has been a large increase in scientific publications on curcuma, used as a food supplement or as an ingredient of functional foods, showing its potential application in several prepathological conditions (Priyadarsini, 2014; Serpa Guerra et al., 2020). This spice was shown to affect several molecular networks influencing each other's, and it may be considered a nutraceutical to be used in a clinical space defined before the drugs, beyond the foods (Li, 2011; Priyadarsini, 2014; Serpa Guerra et al., 2020). *Curcuma longa* L. has long been used also in the treatment of diarrhea, it has a highly pleiotropic interaction with numerous molecular targets involved in inflammation: downregulation of NF-κB; inhibition, via NF-κB, of COX-2, lipoxygenase, and iNOS enzymes; inhibition of inflammatory cytokines, migration inhibitory protein, and PPAR-g (Jobin et al., 1999; Surh et al., 2001; St-Pierre, Couillard, & Van Themsche, 2004). These effects may result in an improvement in gastrointestinal tract functionality of people suffering from inflammatory bowel disease (Holt, Katz, & Kirshoff, 2005; Hanai et al., 2006; Dulbecco & Savarino, 2013). In addition, in ex vivo studies, Curcuma extract exerts a myorelaxant effect on the ileum and colon of

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rats affected by DSS-induced ulcerative colitis due to a direct effect on the intestinal smooth muscle occurring, at least in part, through non-competitive and reversible inhibition of cholinergic muscarinic receptors. Interestingly, these effects are independent of the anti-inflammatory properties and provide the rationale for using *Curcuma Longa* L. in functional motor disturbances of the gastrointestinal tract (Aldini et al., 2012). In addition, the concentration affecting intestinal smooth muscle contractility does not alter this parameter in the bladder, aorta, trachea, and heart of healthy animals (Micucci et al., 2013). Curcumin was shown to preserve colon tissues response to carbachol from colitis induced alterations (Lubbad, Oriowo, & Khan, 2009). In addition, curcumin reduces spontaneous and KCl-induced contractions in isolated rabbit jejunum preparation (Gilani et al., 2005). The inhibitory effects of curcumin were also observed in spontaneous and KCl-induced contractions in Wistar rats (Emami et al., 2020). Furthermore, curcuminoids inhibit acetylcholine and histamine induced contractions in guinea pig ileum (Itthipanichpong et al., 2003). In addition, curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin exert spasmolytic activity in guinea pig ileum precontracted with KCl 60 mM (Jamil et al., 2018). Therefore, we hypothesize that the gastrointestinal effects observed in our study may depend on these molecules. Therefore, the gastrointestinal effect probably due to curcuminoids also deserves a study concerning the possible formulation of functional foods, which is useful for gastrointestinal disorders. In addition, in order to better understand the potential application of turmeric, it is important to investigate smooth muscle contractions, following an innovative method allowing to analyse of the extent and frequency of contractions. We previously demonstrated that these parameters undergo significant alterations in experimental ulcerative colitis. Fast Fourier transforms (FFT) analysis allows studying the contraction frequencies associated with the spontaneous motility of tissues in different conditions. This approach is fundamental to understanding the effects of the different preparations on smooth muscle: low frequencies seem to be associated with the transport of the bolus in the digestive system. Therefore, an increase/decrease in the low-frequency component is related to the transit speed in the specific tract (Shi, Lin, Powell, & Sarna, 2011; Zhang et al., 2016). On the other hand, high frequencies may be associated with an abnormal contraction resulting in poor bolus motility and an increase in painful spasms (Shi et al., 2011; Haraux et al., 2014). In addition to the beneficial effects for gastrointestinal disorders, turmeric may be used in food preservation due to its antioxidant and antimicrobial effects. Thanks to its biological properties, mainly referred to the curcuminoids content, *Curcuma longa* L. may be used as a healthy ingredient of functional foods (Serpa Guerra et al., 2020) We concentrated the present study on the effects related to gallbladder contraction, which have been demonstrated (Abdul Rasyid, Rahman, Jaalam, & Lelo, 2002). While many other phenolic compounds are found in the *Curcuma longa* L. rhizome (Sabir et al., 2021) we focused on curcuminoids considering their potential activity at the gastrointestinal level. The relationship between curcuminoids concentration and in vitro ability of turmeric samples to affect guinea pig biliary tracts, ileum, and colon spontaneous contractility was here studied. Thus, the data reported here could be a starting point for further investigations regarding the application of turmeric based nutraceutical products specific amount/range of curcuminoids in several pathologies characterized by gut motility alterations such as Crohn disease and ulcerative colitis.

2. Materials and methods

2.1. Plants material

The samples consisted of two different organic turmeric powders of *Curcuma longa* L. (C1 and C2), both obtained from organic farming and purchased in a herbal shop. Sample C1 was from non-UE agriculture, while for sample C2 this indication was not provided on the label. The two samples of turmeric powder are commonly used as food additives.

2.1.1. Extraction and HPLC-DAD analysis

Curcuminoids, curcumin (CU), bisdemethoxycurcumin (BDMC), and demethoxycurcumin (DMC), were extracted from turmeric powder by liquid − liquid extraction using methanol as the extractive solvent as indicated by Paramasivam, Poi, Banerjee, & Bandyopadhyay (2009). A reverse phase method was used for the HPLC separation and quantification, according to a modified version of the method suggested by Li et al. (2011). Turmeric powder, 200 mg, was suspended in 10 mL of methanol (CAS 67–56-1, Sigma Aldrich, Germany) and agitated by vortex for 1 min, and total curcuminoids were extracted in an ultrasonic bath at room temperature for 15 min. The procedure was repeated three times. The three methanol fractions were collected and transferred to a 50 mL volumetric flask by methanol. If necessary, it was further diluted to obtain the working solution. The solution was filtered through a nylon filter, 0.45 μm, and 10 μL of the extract was injected into a high-performance liquid chromatography. The HPLC system was an HP1260 series coupled to an auto-sampler Series 1100, an HP1050 diode array detector DAD (Agilent Technologies, Palo Alto, CA), and an analytical column Kinetex 5μ C18 100 Å, 150X4,60 mm (Phenomenex Torrance, CA). The gradient elution was performed by using acetonitrile (CAS 75–05-8, Sigma Aldrich, Germany) and water (Lichrosolv HPLC CAS 7732–18-5, Sigma Aldrich, Germany) with 0.1% of formic acid (CAS 64–18-6, Sigma Aldrich, Germany) as mobile phases A and B, respectively, according to the following gradient: at 0 min, 40% A; from 0 min to 18 min, increased to 50% A; from 18 to 36 min decreased to 0% A, and from 36 to 37 min, increased to 40% A, and kept up to 40 min. A flow rate of 0.4 mL/min was used at room temperature. The detection wavelength was set at 420 nm. Data were acquired with Chemstation for LC3D software (Agilent Technologies, Palo Alto, CA). For each sample, three independent replicates were performed. Curcuminoids were quantified by using an external standard calibration curve with concentration ranges of 0.62–12.33, 0.64–12.79, 0.63–12.56 μg/mL, for curcumin (CAS 458–37-7, Sigma Aldrich, Germany), bisdemethoxycurcumin (CAS 33171–05-0, Sigma Aldrich, Germany), and demethoxycurcumin (CAS 22608–11-3, Sigma Aldrich, Germany), respectively. The linear regression equations and correlation coefficients were calculated for curcumin ($y = 212.53x–56.79$; $r2 = 0.999$), bisdemethoxycurcumin ($y = 224.81x–64.31$; $r2 = 0.999$), and demethoxycurcumin ($y = 228.46x–56.29$; $r2 = 0.999$). The limit of detection (LODs) and limit of quantitation (LOQs) were calculated from the calibration curve based on the standard deviation of the response and the slope as indicated by the ICH Harmonised tripartite guideline. The LODs were 0.25, 0.29, 0.30 μg/mL for curcumin, bisdemethoxycurcumin, and demethoxycurcumin, respectively, whereas their LOQs were 0.50, 0.58, and 0.61 μg/mL. Identification of curcuminoids was performed by comparing their retention times and absorption spectra with those of respective standard solutions (Figure 1 of Supplementary materials).

2.2. Functional studies

2.2.1. Animals

Male guinea pigs (200–400 g) obtained from Charles River (Calco, Como, Italy) were used. The animals were housed according to Directive 2010/63/EU of the European Parliament (following eth-ic-shttps://https://www.wma.net/policies-post/wma-statement-on-animal-use-in-biomedical-research/) and the ARRIVE guideline (McGrath, Drummond, McLachlan, Kilkenny, & Wainwright, 2010). All animal experiments followed the guidelines of the animal care and use committee of the University of Bologna (authorization numbered as “Protocol PR 21.79.14”) and transmitted to the Ministry of Health. Humane end points were followed (https://www.humane-endpoints.info/en). Immediately after sacrifice by cervical dislocation, the gallbladder, bile distal duct, sphincter of Oddi, ileum, and proximal colon tracts were excised and set up rapidly under a suitable organ bath as previously

described (Micucci et al., 2014; Micucci et al., 2020).

2.2.2. In vitro spontaneous contractility

The tracing graphs of spontaneous contractions (SC) (g/min) of ileum, colon, gallbladder, and gastric fundus were continuously recorded with LabChart Software (version 5.04, GraphPad Software Inc., San Diego, CA, USA). The extracts used in the experiments were made with the following procedures: turmeric powder, 200 mg, was suspended in 10 mL of methanol (CAS 67-56-1, Sigma Aldrich, Germany) and agitated by vortex for 1 min. The procedure was repeated three times. The methanol was evaporated under vacuum, and the residue was weighted and dissolved in DMSO. After the equilibration period (about 30 min to 45 min according to each tissue), cumulative-concentration curves (0.1, 0.5, 1, 5, 10 mg/mL) of turmeric extracts were exerted. Data were reported as percentage values of basal spontaneous contraction activity: zero represented the basal tone, and each point (mean ± SEM) was the per cent variation from the baseline after the cumulative addition of each dose of C1 and C2. Statistical analysis was performed by one sample *t*-test or ANOVA followed by Bonferroni post hoc test, as appropriate (GraphPad Prism version 5.04, GraphPad Software Inc., San Diego, CA, USA). In all comparisons, the level of statistical significance (P) was set at 0.05. All experiments were performed in triplicate. For each turmeric concentration, over a 5 min interval, the following parameters were evaluated: mean contraction amplitude (MCA), calculated as the mean force value (g); the force contractions standard deviations, considered as an index of the spontaneous contraction variability (SCV); and basal spontaneous contraction activity (BSCA), calculated as the percentage (%) variation of each mean force value (g) with respect the control. The spontaneous contractions rates were evaluated through standard FFT analysis and evaluation of the absolute powers of the following frequency bands of interest performed (expressed in Hz): [0.0,0.1]; [0.1,0.2]; [0.2,0.3]; [0.3,0.4]; [0.4,0.5]; [0.5,0.6]; [0.6,0.7]; [0.7,0.8]; [0.8,0.9]; [0.9,1.0]. To simplify data presentation, the powers were summed over greater frequency intervals, and the following classification was introduced: low [0.0,0.2], medium [0.2,0.6], and high [0.6,1.0] frequency (Micucci et al., 2020). All calculations were performed in a post-processing phase with Lab Chart Software. To avoid errors due to the presence of artefacts, the period of analysis was chosen by a skilled operator who was blinded to the treatment.

3. Results

3.1. Chemistry

The results obtained are expressed as a percentage g of curcuminoids per 100 g of turmeric root powder, as shown in Table 1.

In the analysed samples, curcumin was the most represented compound, followed by DMC and BDMC, which were always found in smaller quantities. In sample C1, higher curcuminoid contents were found, almost twice as much as in sample C2, thus confirming data in the literature reporting the highly variable curcuminoid content of the cultivars of C. *longa* (Thomas, Zachariah, Syamkumar, & Sasikumar, 2011). Considering the different amounts of curcuminoids, the extrapolated final concentrations of BDMC, DMC, and CU used in the in vitro studies are detailed in Table 2.

Table 1
Curcuminoids content in turmeric powder[1].

***Curcuma Longa* L. powder**[2]	**BDMC**	**DMC**	**CU**	**Total Curcuminoids**
C1	0.64 ± 0.03 (4.37)	0.83 ± 0.01 (1.43)	2.10 ± 0.02 (1.17)	5.22 ± 0.06
C2	0.30 ± 0.01 (3.76)	0.36 ± 0.01 (1.42)	0.91 ± 0.01 (0.89)	2.31 ± 0.03

[1] The curcuminoid content is expressed as a percentage g of curcuminoids per 100 g of tur-meric root powder. Results are presented as mean ± standard deviation. Moreover, for BDMC, DMC and CU, the RSDr values are also reported. CV (in brackets): Coefficient of Variation % = (Standard deviation/mean)*100. The CV is derived from the following formula: RSDr [(Sr / $\bar{x}$) × 100].

[2] Turmeric root powder (raw organic). BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CU, curcumin.

3.2. Spontaneous contractions in vitro studies

3.2.1. Gallbladder

The maximal increase in gallbladder tone was comparable for C1 (~10%) and C2 (~15%), constant over concentration for C1, and biphasic for C2, although within a small range of variation. Therefore, the increase in gallbladder tone did not seem to be concentration-dependent, especially considering that C1 contains more than twice the amount of curcuminoids than C2. This suggests that the compounds responsible for the observed effect have reached their maximum effect at the lowest concentration used, since increasing the amount of *Curcuma longa* L. powder up to 10 mg/mL, gallbladder tone was mostly unchanged. An example of an experimental recording of the concentration–response curves of C1 and C2 on spontaneous gallbladder basal contractility is reported in Figure 2 Supplementary material.

The basal differences are due to the intra- and inter-variability of the animals. Considering the experimental concentration–response curves, increased doses of C1 and C2 did not induce any changes in spontaneous contractions (SC); the gallbladder spontaneous contractility profiles showed that increasing doses of turmeric samples augmented the mean contraction amplitude of muscular tone by 10% (C2) and 5% (C1). Wavelength changes were also observed, but absolute band powers measured after the addition of each concentration of C1 and C2 were not dose-related. Fig. 1 depicts two different experiments: therefore, excluding the control (for which the variability is very different), the contractility for C1 and C2 is similar.

3.2.2. Distal bile duct

Spontaneous contraction increased in a dose-dependent manner for C1 and C2 from the lowest concentrations (0.1 mg/mL) up to the concentration of 1 mg/mL, and it remained constant thereafter for both Curcuma samples, suggesting that the maximal effect was achieved at

Table 2
Curcuminoid content in the solutions used for in vitro studies.

***Curcuma Longa* L. powder**	Solutions (mg/mL)	**BDMC** μg/mL (μM)	**DMC** μg/mL (μM)	**CU** μg/mL (μM)	**Total** μg/mL
C1	0.10	0.64 (2.07)	0.83 (2.45)	2.10 (5.70)	5.22
	0.50	3.20 (1.04)	4.15 (12.26)	10.50 (28.50)	26.10
	1.00	6.40 (20.70)	8.30 (24.50)	21.00 (57.00)	52.20
	5.00	32.00 (10.40)	41.50 (122.60)	100.50 (285.00)	261.00
	10.00	64.00 (207.00)	83.00 (245.00)	210.00 (570.00)	522.00
C2	0.1	0.30 (0.97)	0.36 (1.06)	0.91 (2.47)	2.31
	0.50	1.50 (24.32)	1.80 (5.32)	4.55 (12.35)	11.55
	1.00	3.00 (9.70)	3.60 (10.60)	9.10 (24.70)	23.10
	5.00	15.00 (243.20)	18.00 (53.20)	45.50 (123.50)	115.50
	10.00	30.00 (97.00)	36.00 (106.00)	91.00 (247.00)	231.00

BDMC (bisdemethoxycurcumin), MW 308.3; DMC (demethoxycurcumin), MW 338.4; CU (curcumin) MW 368.4.

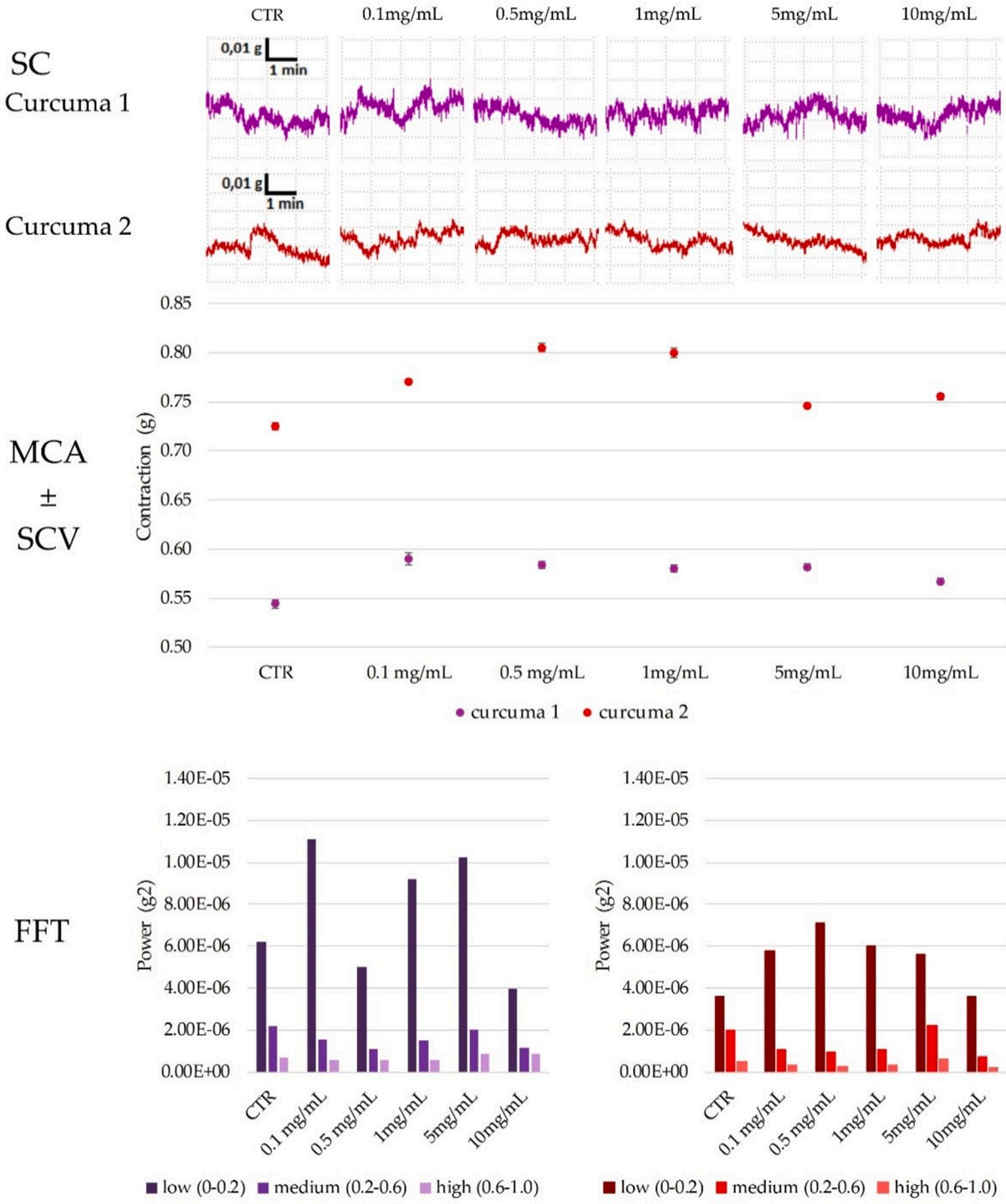

Fig. 1. Experimental original recording of the concentration–response curve of C1 and C2 on spontaneous gallbladder basal contractility. Spontaneous contraction (SC); spontaneous contraction variability (SCV); mean contraction amplitude (MCA); spontaneous contraction rates (FFT).

1 mg/mL (Figure 3 Supplementary material). Focusing on the fact that two different preparations of the distal part of the common bile duct were used, it was observed that the differences from the control conditions were similar; the contractility pattern was not altered (SC), while there was a similar increase in MCA values.

Even if the different basal values were different, there was an increase in MCA for C1 and C2 of 5% and 10%, respectively. Absolute band powers of control and treated tissues (FFT) showed that the wave bands increased after C2, but not after C1, consistent with a higher increase in MCA for C2 than for C1 (Fig. 2).

3.2.3. *Sphincter of Oddi*

The Oddi sphincter tone was not affected by either Curcuma sample (Figure 4 Supplementary material). In agreement with previous results, the contractility pattern waves were not affected by either Curcuma samples; the per cent variation of MCA was similar in different basal conditions. In the C2 experiment, starting from basal conditions with very low variability, turmeric did not produce any changes. In the experiments of C1, on the other hand, starting from conditions characterized by a higher variability, at the concentration of 0.1 mg/mL, the oscillations increased, while they decreased at higher concentrations

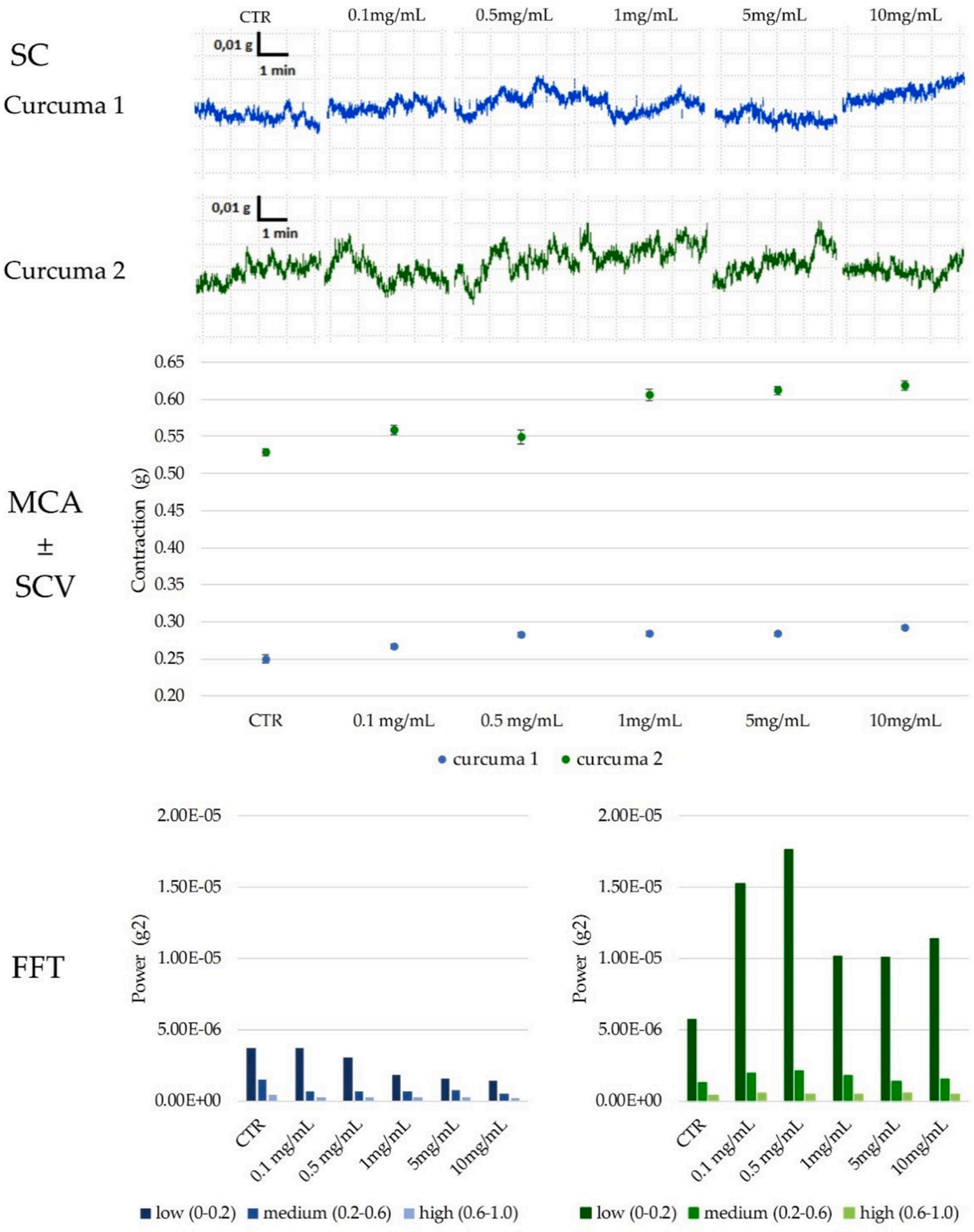

Fig. 2. Focus on experimental original recording of the concentration–response curve of sample C1 and C2 on spontaneous distal bile duct basal contractility. Spontaneous contraction (SC); spontaneous contraction variability (SCV); mean contraction amplitude (MCA) and spontaneous contraction rates (FFT).

(Fig. 3).

3.2.4. *Ileum*

As reported in Figure 5 of the Supplementary material, C1 and C2 decreased the basal spontaneous contraction activity in a concentration-dependent manner.

The effect was more pronounced for C2 than for C1, even if in C2 the total amount of curcuminoids is half that of C1, while the maximal C1 and C2 concentrations (10 mg/mL) decreased ileal tone by 40%. Since sample C1 has twice the concentration of curcumin than C2, and the pattern is similar, there was a decrease in the tone up to 1 mg/mL, and it then remained constant. For Curcuma C2, the effect occurred at half the concentration compared to c1, with remarkable differences: for each point, curcuminoids from Curcuma C2 are half of those of C1. At lower

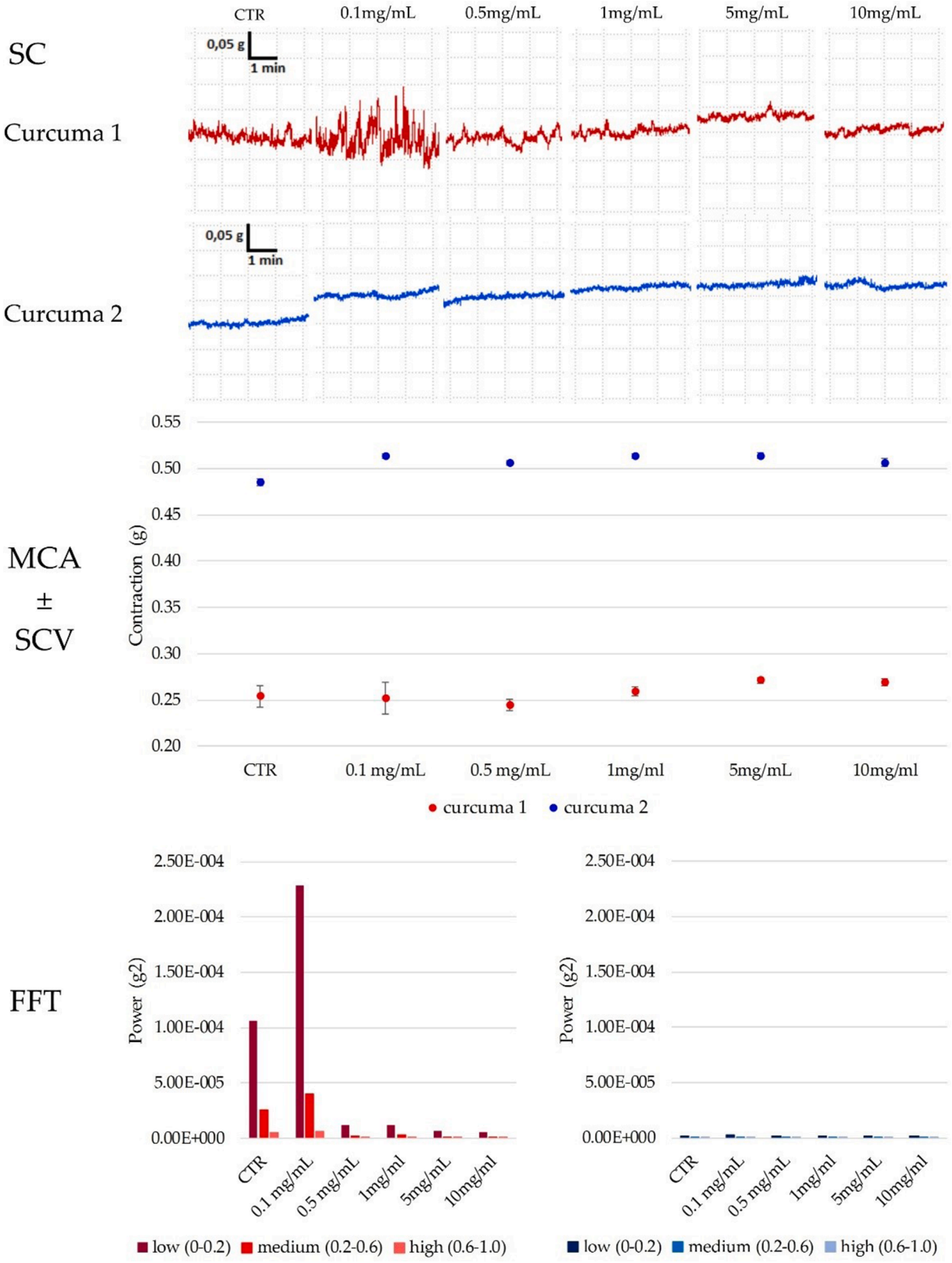

Fig. 3. Focus on experimental original recording of the concentration–response curve of C1 and C2, on spontaneous sphincter of Oddi basal contractility. Spontaneous contraction (SC); spontaneous contraction variability (SCV); mean contraction amplitude (MCA); spontaneous contraction rates (FFT).

doses of Curcuma, the contraction pattern was unchanged, while at higher doses, it was reduced. MCA decreased in a dose-dependent manner, and the effect was more evident for sample C2 (from 1.68 g to 0.9 g) than for C1 (from 0.50 g to 0.30 g). The same was shown by FFT values that were almost abolished, mainly by C2, despite a paradoxical increase at the lower dose (0.1 mg/mL): Sample C2 started from conditions of greater basal variability. The oscillations increased at the concentration of 0.1 mg/mL and they decreased until they disappeared

at concentrations higher than 1 mg/mL. C1 did not modify the basic oscillations up to 0.5 mg/mL, while it strongly reduced them at higher concentrations (Fig. 4).

3.2.5. Colon

Basal spontaneous contraction activity decreased in a dose-dependent manner, mainly for C1. In fact, C1 reduced the basal spontaneous contraction activity in a dose-dependent manner, with a

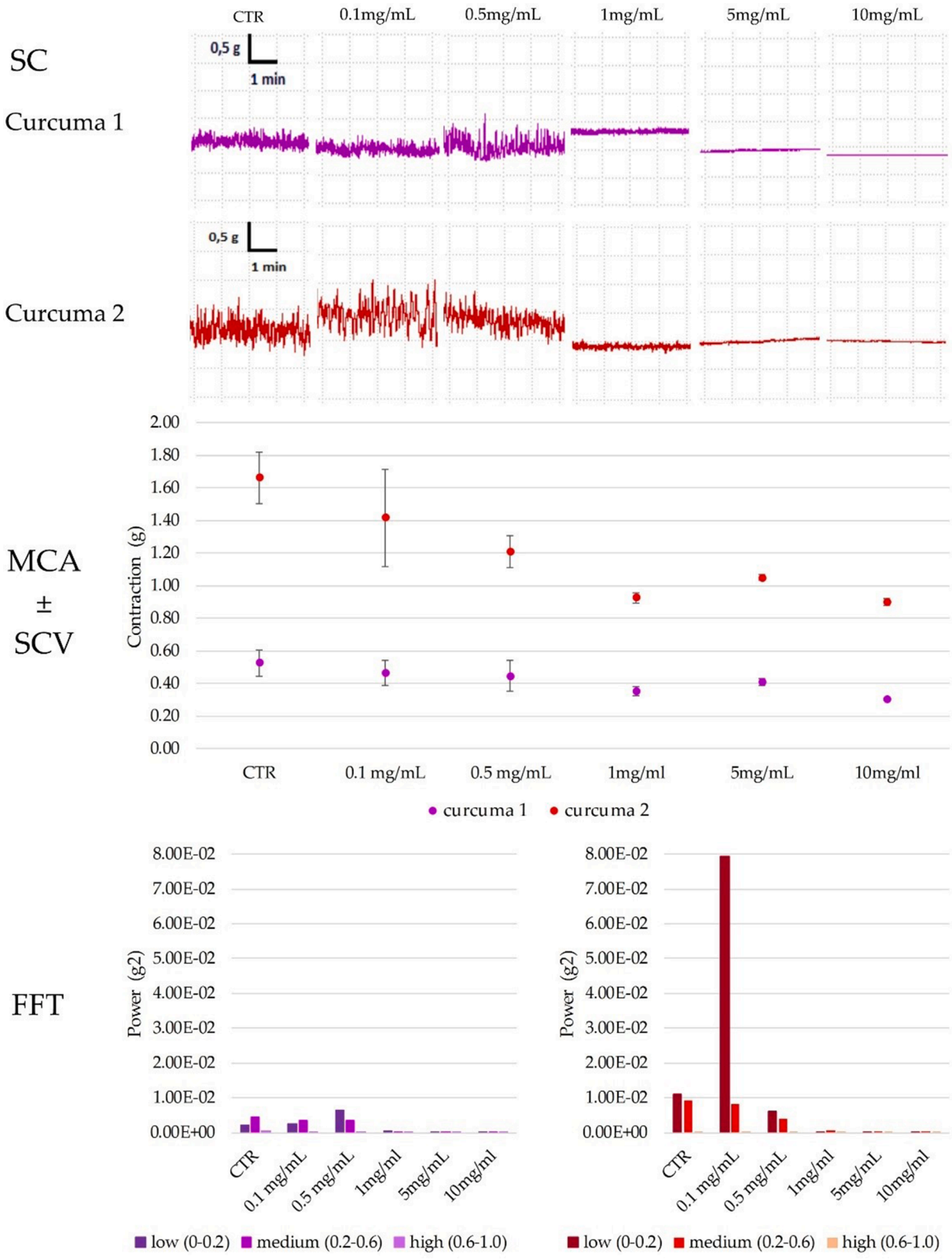

Fig. 4. Focus on experimental original recording of the concentration–response curve of C1 and C2 on spontaneous ileum basal contractility. Spontaneous contraction (SC); spontaneous contraction variability (SCV); mean contraction amplitude (MCA); spontaneous contraction rates (FFT).

maximum effect of −44% at 10 mg/mL. C2 had a weaker effect on ileal tone, plateauing at 20% of the decreasing effect for concentrations higher than 1 mg/mL (Figure 6 Supplementary material).

The net effect on colon tone was higher for C1. According to the previous results, MCA values decreased with doses from the baseline value for C1 (from 0.40 g to 0.20 g) and for C2 (from 0.55 g to 0.44 g), thus confirming that C1 is more effective in decreasing colon contractility. Each turmeric sample reduced fluctuations from the concentration of 1 mg/mL. Since the effects of C2 were on a colonic specimen with baseline conditions of greater variability, the reductions were more significant.

However, it is noteworthy that with C2, at the concentrations of 0.1 and 0.5 mg/mL, the oscillations in the medium frequencies increased while they were reduced drastically at higher doses (Fig. 5).

4. Discussion

Several authors have quantified curcuminoids in powders of *Curcuma longa* L. finding values in the intervals of 0.29–0.73%, 0.20–0.71%, and 0.39–2.16% referred to BDMC, DMC and CU, respectively, with total curcuminoid contents in the range 0.96–3.6% (Kulyal et al., 2016), while Ali, Haque, & Saleem (2014) reported contents of 0.1–0.46–2.12% for BDMC, DMC, and CU respectively. A study on turmeric rhizome powder on samples from various locations in Thailand found total curcuminoid contents between 3.07 and 9.58% (Pothitirat & Gritsanapan, 2006). The total curcuminoid contents in the samples analysed here are in line with those reported in the literature. Given the high variability of the curcuminoid content, it is thus essential to quantify these active molecules, which are highly relevant for the quality of turmeric, mainly if it is used for nutraceutical or phytotherapeutic purposes, where the use of titrated extracts is necessary. In fact, as reported by Nasef et al. (2019), curcuminoids in the presence of turmeric and in some foods may have higher bioavailability than the relative purified ones. Several inflammatory diseases result in alterations of many parameters, including gut motility. Turmeric anti-inflammatory properties have been widely demonstrated in preclinical and clinical trials and shown to be related to diarhyleptanoids concentration, however, the relationship between curcuminoids concentration and turmeric effects on smooth muscle intestinal contractility has not yet been investigated. This study aimed to investigate whether the amount of curcuminoids in turmeric may affect gut smooth muscle contractility.

The two samples of *C. longa* L. used were obtained from two different organic farms and showed different curcuminoid contents: C1 had more than double the content of total curcuminoids in C2. As reported in Table 1, C1 has 5.22% of total curcuminoids, while C2 has 2.31%. Many reports have highlighted that curcumin content varies within accessions of *C. longa* L. and from place to place due to the influence of environment and agroclimatic conditions (Thomas et al., 2011; Sheeja, Deepa, Santhi, & Sasikumar, 2015), and to the different expression levels of genes encoding important enzymes of the curcuminoid biosynthetic pathways (Katsuyama, Kita, Funa, & Horinouchi, 2009; Sheeja et al., 2015).

C1 and C2 effects towards the gastrointestinal system were investigated. As these extracts reduce colon and ileum smooth muscle contractility, their influence on the biliary tract was also tested. Indeed, bile duct dilatation is associated not only with different pathological conditions, such as congenital biliary dilatation, Caroli's disease, choledocholithiasis, gallbladder cancer, but also with some medical treatments, such as opiate drugs, that decrease ileum and colon contractility (Smyth & Stace, 2016) and ketamine (Yu et al., 2014). C1 and C2 were shown to decrease colon and ileum smooth muscle contractility, to slightly favour gallbladder and distal bile duct contractions, without affecting Sphincter of Oddi contractility, therefore they may exert a positive effected in subjects affected by pathologies characterized by intestinal hypermotility, without increasing the risk of biliary tract alterations. In addition, we studied their effects in a concentration range between 0.1 mg/mL and 10 mg/mL, corresponding to amounts of curcumin in a range between 2.10 μg/mL and 210 μg/mL for C1, 0.91 μg/mL and 91 μg/mL for C2, corresponding to higher concentrations than those reached in vivo by the administration of high doses of curcumin. These mixtures exert weak effects at high concentrations of curcumin, which are generally not obtained in vivo, suggesting a good toxicological profile (Rasyid & Lelo, 1999; Rasyid, et al., 2002). The effects of Curcuma powder samples C1 and C2 are not different on the gallbladder and bile duct, being almost negligible: they led to a minimal increase in tone. The results on the gallbladder agree with those of Kline & Karpinski (2015), who have shown that none of the curcumin concentrations used (25–100 mM) affected the tension of guinea pig gallbladder strips in basal conditions, suggesting the lack of a cholecystokinetic effect. However, the same study reported that curcumin relaxes both cholecystokinin octapeptide-(CCK) and KCl-induced contraction of guinea pig gallbladder strips in a concentration-dependent manner. The curcumin-induced relaxation effect is mediated by multiple signalling pathways involving the Protein kinase C second messenger system, the inhibition of extracellular Ca^{2+} entry and K^{+} channels. The relaxant effect on gallbladder muscular strips was obtained at concentrations of 25–100 mM, which are much higher than the maximal concentrations used in our studies (0.6 mM); for this reason, the possibility that higher concentrations of C1 and C2 may reduce guinea pig gallbladder CCK- and KCl-induced contractions can not be ruled out. Therefore, we can conclude that curcuminoids relax gallbladder muscle in a stimulated state but not in a basal state. The sphincter of Oddi did not undergo a tone increase. However, C1 decreased the FFT, while C2 maintained the normal wave pattern. It is very important to consider that there is no increase in tone in basal conditions, which would lead to an obstacle to bile flow. On the contrary, Rasyid & Lelo (1999), in a single-blind crossover study, showed that a single oral administration of 20, 40, and 80 mg of curcumin stimulated contraction of the human gallbladder, but no linear relationship was found between doubling of curcumin dosage and doubling of gallbladder contraction. It is important to outline that even in the present study, no linear relationship between curcumin dosage and gallbladder contraction was found. In fact, substantial differences between the two Curcuma samples, with different curcuminoid content, were not observed. The two reported studies may seem to disagree. However, it is essential to consider that the first paper (Kline & Karpinski, 2015) is an in vitro study on the activity of isolated curcumin, in which neurohumoral regulatory mechanisms of gastrointestinal contraction and motility are not operating; while the second Abdul Rasyid et al. (2002) is an in vivo human study, where curcuma, given orally, can trigger neuroendocrinal and humoral mediators, potentially resulting in cholecystokinetic action.

Curcumin is metabolized differently in the mammalian body, depending on the administration route (oral, intravenous or intraperitoneal) (Dulbecco & Savarino, 2013). Curcumin, when orally administered, undergoes glucuronidation and sulfation (Asai & Miyazawa, 2000). Furthermore, in tissues from surgery patients administered high doses of curcumin, no curcumin or very low concentrations of curcumin conjugates were detected. Therefore, the possibility of metabolic degradation or condensation strongly suggests that research should also focus on these molecules rather than only on curcumin itself. Most studies indicate that curcumin glucuronides and tetrahydrocurcumin are less active than curcumin (Pfeiffer et al., 2007; Sandur et al., 2007) however, other data suggesting these metabolites may be more active were published (Pfeiffer et al., 2007). Curcuma C1 and C2 decreased the ileal tone up to 40% at the maximal concentration because it was effective at half the concentration of Curcuma C1. Therefore, at the dose of 1 mg/mL, Curcuma C2 reached its maximal effect. The wave pattern was reduced, like also ileal contractility. Yu et al. (2017) reported that the amplitude of the spontaneous contractile waves of jejunum was reduced in a concentration-dependent manner when normally isolated jejunum of mice was incubated with 10–40 μM of curcumin, a concentration comparable to that used in the present study (see Table 2). They

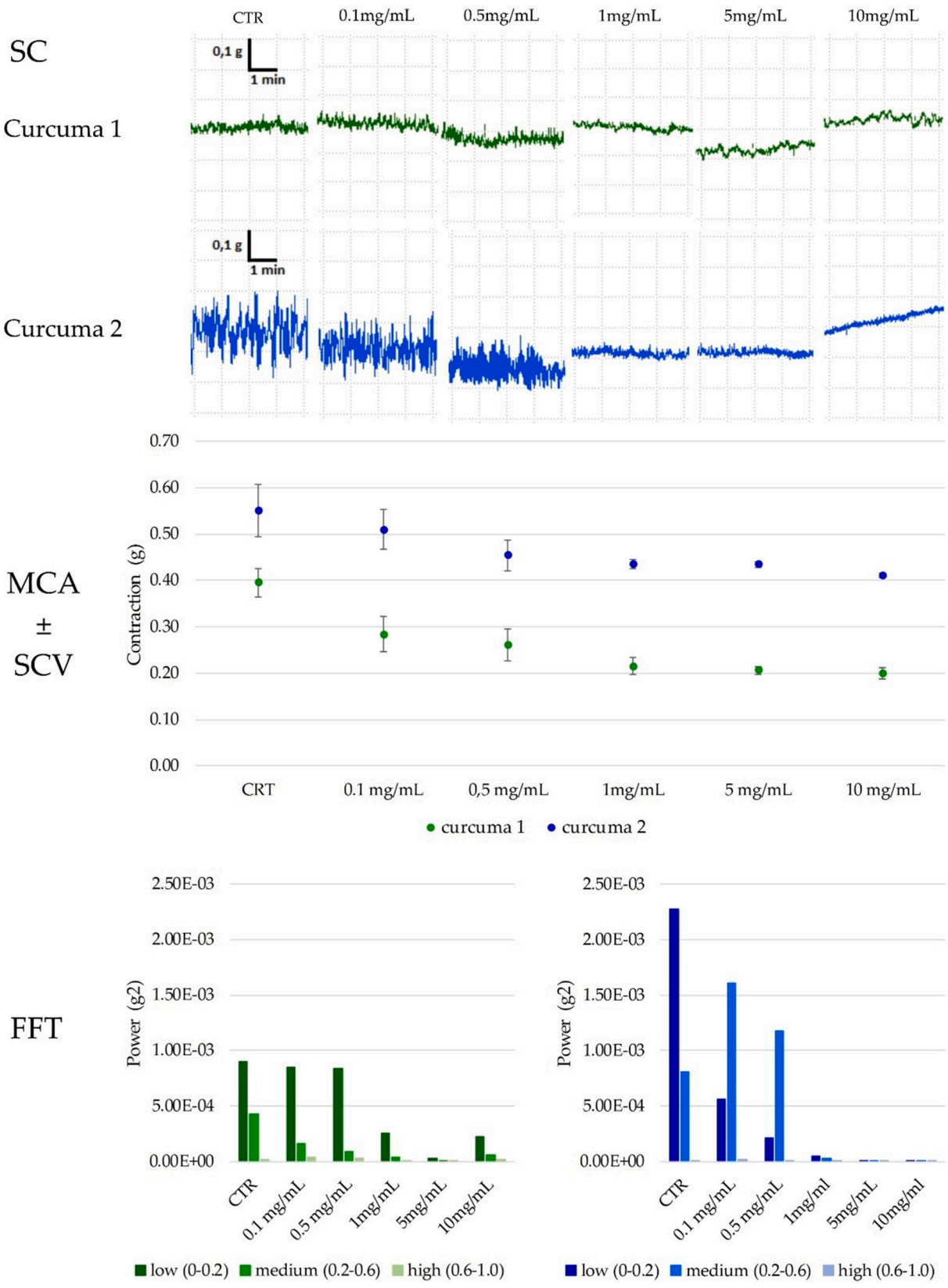

Fig. 5. Focus on experimental original recording of the concentration–response curve of C1 and C2, on spontaneous colon basal contractility. Spontaneous contraction (SC); spontaneous contraction variability (SCV); mean contraction amplitude (MCA); spontaneous contraction rates (FFT).

also found that a moderate dose of curcumin by intragastric administration for more than 10 days may inhibit functional gastrointestinal disorders in mice without affecting normal gastrointestinal transit. Moreover, Kumar, Shrivastava, Purwar, & Arora (2012) have shown that curcumin (2 g/kg intragastrical) decreases intestinal motility in albino rats, and this may partly explain the traditional use of curcumin in disorders like diarrhoea, abdominal cramps, and irritable bowel syndrome. In humans, curcumin improves the clinical outcome of patients with non-alcoholic fatty liver disease (Chashmniam et al., 2019). In addition, the daily administration of curcumin (3.6 g) has benefits in patients with colorectal cancer (Garcea et al., 2005). C1 was more effective than C2 in decreasing colon tone (by 44%, vs 20% at 1.0 mg/mL respectively). MCA and the index of SCV decreased at a maximum Curcuma concentration of 1 mg/mL, but C1 had a 10% higher effect; C1 and C2 decreased the wavelength amplitude. Therefore, C1 had a greater myorelaxant effect on colon contractility in basal conditions, suggesting that a higher curcumin content may lead to colon myorelaxation in fasting conditions. In ileum, curcuma C2 decreased tone more than curcuma C1. However, the spasmolytic efficacy in different tracts remains unclear (Zhang et al., 2016), and the ability to affect contractility in different gastrointestinal tracts with different potencies provides a possible guideline for clinical applications. Gastrointestinal motility is highly complex and comprises fasted and fed (postprandial) motility patterns occurring over hours. The frequency of motility at a specific intestine site is directly related to the slow-wave frequency. The effect of the two formulations is not quite different from each other. The commercially available curcuminoid mix (mixture of Cur, DMC, and BDMC) is more active than curcumin in suppressing TNF-induced NF-κB activation in a wide variety of tumour cell lines, and the relative activity is in the order Cur > DMC > BDMC (Sandur et al., 2007). Curcumin also reduces ATP-stimulated Ca^{2+} mobilization from intact HL-60 cells, suggesting its cell-permeant effect. However, since it also affects intracellular Ca^{2+} pumps and possibly ryanodine receptors, it may lead to complex Ca^{2+} transient responses within cells, which may explain some of its putative therapeutic properties (Dyer et al., 2002). Considering the healthy properties of turmeric and the request of a health claim from the EFSA for a specific property, it may be relevant to promote its introduction into foods (e.g. oils, baked food products) both to enhance their sensory properties and for their beneficial effects. In fact, it would be difficult to exceed the dosages recommended by EFSA, in consideration of the fact that the acceptable daily intake (ADI) is very high (3 mg/kg bw/day, which corresponds to about 200 mg for a person weighing 70 kg), and the intake of curcumin from the diet is generally less than the 7% of the ADI (Additives & Ans, 2010; EFSA, 2017).

5. Conclusions

Two Curcuma formulations with different curcuminoid content reduced, in vitro, guinea pig ileum and colon tones and spontaneous contractility, while (at curcumin concentrations of 0.6 mM) they did not alter gallbladder, bile duct, or sphincter of Oddi contractility (Fig. 6). In particular, turmeric powder C1, containing approximatively twice the amount of curcuminoids than C2, reduced spontaneous colon contractility more than turmeric powder C2, and the effect occurred in a concentration-dependent manner. On the ileum, both turmeric powders C1 and C2 reduced the tone, but C2 was more effective, despite the lower amount of curcuminoids. The reduction of low-frequency waves may be associated with a decrease in bolus transit speed in specific anatomical tracts. This effect may lead to a gastrointestinal functionality improvement if it occurs in intestinal tracts where it induces better digestion resulting from an increase in mixing, degradation, and absorption., However, it may negatively impact the functionality of other intestinal tracts. High-frequency waves are related to painful spasms; thus, their decrease may determine a positive effect. After gallbladder emptying, bile reaches the bile duct where the effects of C1 and C2 are different: C1 may delay the transit while C2 may increase its speed (C2 effects are not significant). The bile crosses the sphincter of Oddi where C1 may reduce transit time (C2 has no significant effect). Both C1 and C2, at different threshold concentrations, may increase the transit time of the food bolus, favouring digestive processes in both the ileum and colon.

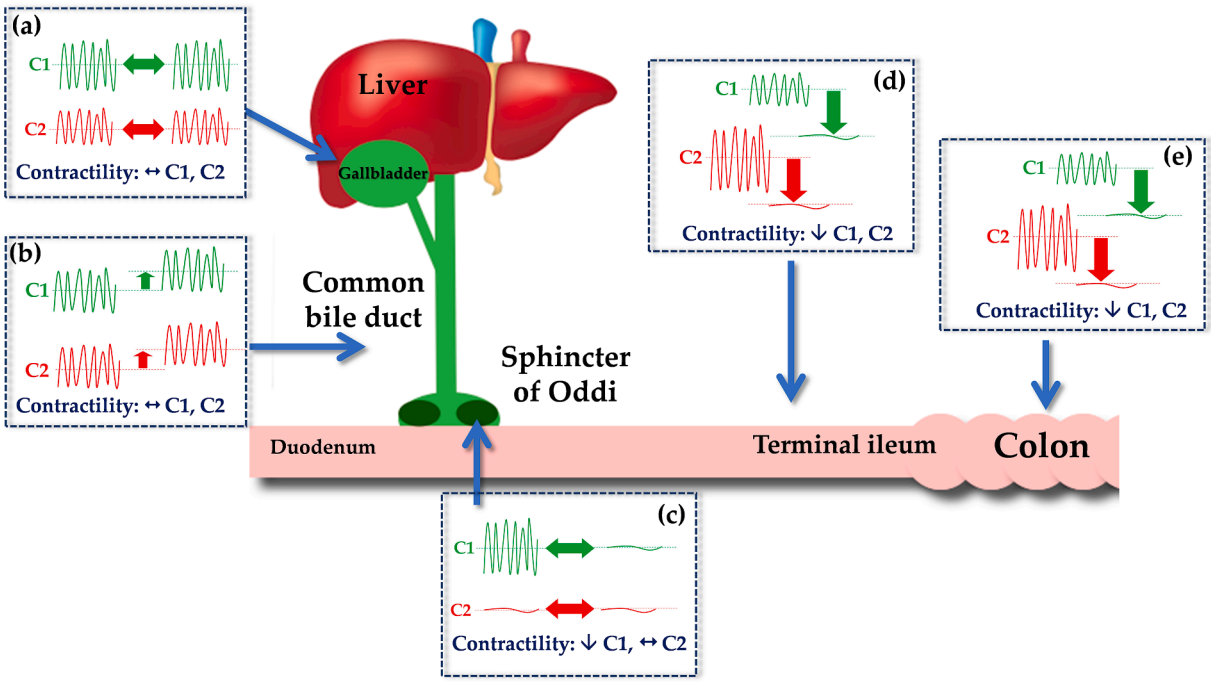

Fig. 6. Summary of the effects of curcuma extracts. (a) Gallbladder: no differences in tone and spontaneous contractility; (b) Bile duct: tones increased with both C1 and C2 but contractility does not change; (c) Sphincter of Oddi: tones are quite similar but a marked reduction in contractility with C1 was present (the basal contractility of the samples treated with C2 was very low and no modifications were seen); (d) Ileum: C1 and C2 reduced the tones (more for C1) and contractility; (e) Colon: both C1 and C2 reduced the tones and spontaneous contractility. Both C1 and C2 induced biomechanical effects in ileum and colon without affecting the behavior of secondary tissues (gallbladder bile duct and sphincter of Oddi).

Phytocomplexes seem to affect several biological functions, affecting several molecular networks, thus, it is difficult to ascribe the observed effects to isolated compounds. However, it may be useful to identify the molecules mainly involved in modulation of specific functions. In this case, we can conclude that the amount of curcuminoids may be relevant for the formulation of functional foods and nutraceuticals, mainly acting in specific anatomical regions. In fact, we have observed that, in specific gastrointestinal tracts, turmeric-based preparations with different concentrations of the three studied curcuminoids determine different effects, like in the case of the colon and ileum. Consequently, our data could be considered for the development of formulations with different concentrations of specific curcuminoids, basing on the anatomical tracts to be targeted and on the effects to be determined. For example, in pathological conditions characterized by diarrhoea, such as ulcerative colitis, the administration of a turmeric based preparation with a higher concentration of curcuminoids may be more effective than a preparation with lower curcuminoids concentrations. For the formulation of specific functional foods or nutraceuticals, further studies are needed to identify the more targeted composition of turmeric for any of the cited gastrointestinal pathologies.

Ethical statement

The study was conducted according to the guidelines of the 504 Declaration of Helsinki, and approved by the Committee of the University of Bologna (Protocol PR 505 21.79.14) and transmitted to the Ministry of Health.

CRediT authorship contribution statement

Matteo Micucci: Methodology, Data curation, Writing – original draft, Writing – review & editing. **Roberta Budriesi:** Conceptualization, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Mara Mandrioli:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Matilde Tura:** Data curation, Writing – original draft, Writing – review & editing. **Ivan Corazza:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Maria Frosini:** Methodology, Data curation, Writing – review & editing. **Rita Aldini:** Writing – review & editing, Supervision. **Laura Beatrice Mattioli:** Methodology, Investigation. **Tullia Gallina Toschi:** Conceptualization, Resources, Data curation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105314.

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