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Valentina Azzurra Papillo[a], Marco Arlorio[a], Monica Locatelli[a], Laura Fuso[b], Nicoletta Pellegrini[c,d*], Vincenzo Fogliano[d]

[a] Università degli Studi del Piemonte Orientale "A. Avogadro" – Dipartimento di Scienze del Farmaco– Largo Donegani 2, 28100 Novara, Italy

[b] M.I.A.C. S.c.p.a.- Polo AGRIFOOD Regione Piemonte, Dronero (Cuneo, Italy)

[c] Department of Food and Drug, Human Nutrition Unit, University of Parma, Parco Area delle Scienze 47/A, 43124 Parma, Italy

[d] Food Quality and Design Group, Wageningen University and Research Centre, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

*Corresponding author at: Department of Food and Drug, Human Nutrition Unit, University of Parma, Parco Area delle Scienze 47/A, 43124 Parma, Italy

e-mail address: nicoletta.pellegrini@unipr.it

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Abstract

Pharmaceutical formulations for improving stability and bioavailability of curcuminoids are often produced using excipients unsuitable for food applications. In this work, turmeric oleoresin was microencapsulated by spray drying using gum arabic and maltodextrins to prepare a new ingredient (GA/MD) for food industry. *In vitro* bioaccessibility and degradation of curcuminoids along the gastro-intestinal tract was investigated, and compared with two commercial ingredients, turmeric powder and Meriva®. Curcuminoids were significantly degraded under the gastro-intestinal conditions in all the formulations; however, their bioaccessibility in GA/MD ingredient was 25-fold higher than that of turmeric powder, but slightly lower (1.2-fold) to that of Meriva®. After addition to rice and yoghurt, the curcuminoid bioaccessibility of GA/MD was about 2- fold higher than for the ingredient alone and 1.5- fold higher than for Meriva®; furthermore, addition to rice improved bioaccessibility more than in yogurt. Studies using different coating agents and other food matrices are needed.

Keywords

Microencapsulation; spray-drying; food matrix effect; turmeric; bioaccessibility; Twin-SHIME® dynamic model.

1 Introduction

Turmeric (*Curcuma longa* L.) is a member of *Zingiberaceae* family cultivated in tropical and subtropical regions around the world. It is a rhizome extensively used as spice, traditional medicinal herb, food coloring and additive (E100) (Kocaadam & Sanlier, 2017) as well as, more recently, ingredient in food supplements. The main colored and bioactive compounds in turmeric are the curcuminoids: the curcumin 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), the demethoxycurcumin and the bisdemethoxycurcumin. They belong to the group of diarylheptanoids (or diphenylheptanoids) having an aryl-C7-aryl skeleton (Li et al., 2011). Curcuminoids are considered the main active and abundant ingredients in turmeric; it has shown antitumor, antioxidant, antimicrobial and anti-inflammatory properties both in *in vitro* and in *in vivo* studies (Kocaadam et al., 2017).

The World Health Organization stated that the acceptable daily intake of curcuminoids as food additive is in the range of 0-3 mg/kg (Amalraj, Pius, Gopi & Gopi, 2017). An average daily intake of turmeric in the Indian diet (largely devoted to the use of turmeric) was estimated of approximately 60-100 mg of curcuminoids (Mahmood, Zia, Zuber, Salman & Anjum, 2015).

Curcumin is industrially produced from turmeric oleoresin, which is a brownish-orange viscous oily product extracted from the rhizome generally using a non-aqueous solvent (mainly ethanol, methanol, acetone, isopropanol, dichloromethane or hexane), followed by the removal of the solvent by evaporation (Jayaprakasha, Negi, Anandharamakrishnan, & Sakariah, 2001). Turmeric oleoresin is not water-soluble and contains resinous material, volatile compounds, essential oils (Hastak et al., 1997) and about 30-45% (w/w) of curcuminoids (Kshirsagar, Yenge, Sarkar, & Singhal, 2009), the concentration of which depends on variety, agronomical practices (Li et al., 2011) and extraction methods.

A downside of curcumin is represented by its low stability when exposed to light, high temperature, metallic ions, enzymes and oxygen (Wang, Lu, Lv, & Bie, 2009). It is also characterized by a low bioavailability, related to low bioaccessibility, high rate of metabolism, rapid elimination and clearance from the body (Lee et al., 2013). The low bioaccessibility, in turn, depends on very limited water-solubility (18 ng/mL) (Kaminaga et al., 2003), that also particularly depends on the molecular behaviour, because of the keto-enol forms due to the tautomerism which is able to influence the hydrophobicity and polarity. Moreover, curcuminoids are susceptible to degradation under alkaline conditions, reducing additionally the bioavailability. Particularly, at pH above 7 and within thirty minutes, it degrades to trans-6-(40-hydroxy-30-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane, and vanillin (Wang et al., 1997). Conversely, under acidic conditions, the degradation of curcumin is slower, with less than 20% of total curcumin decomposed in 1 h (Wang et al., 1997). Therefore, when curcumin is orally ingested, only a small portion is absorbed within the intestine, metabolized and then excreted through urine (Hoehle, Pfeiffer, Solyom, & Metzler, 2006). The major portion of curcumin arrives to the colon and it is partially metabolised by colonic bacteria. In any case, more than 75% of curcumin is excreted in faeces (Lee et al., 2013).

Many physical strategies have been developed to improve stability and bioavailability of curcumin, including its inclusion in liposomes (De Leo et al., 2018; Pu, Tang, Li, Li, & Sun, 2019), nanoparticles and polymeric micelles, micro- and nano-emulsions (Araiza-Calahorra, Akhtar, & Sarkar, 2018) and phospholipid-complexes (Liu et al., 2016). The latter approach was applied in Meriva®, a patented phytosome-like ingredient used in food supplements marketed in the USA and Europe (Belcaro et al., 2010). Formulation of curcumin with adjuvants like piperine and quercetin (Anand, Kunnumakkara, Newman, & Aggarwal, 2007), or with volatile oils present in oleoresin, led to an increase of curcumin absorption in humans (Jager et al., 2014). Furthermore, food matrix and cooking method may affect the bioaccessibility and bioavailability of curcuminoids (Zou, Liu, Liu, Xiao, & McClements, 2015; Vitaglione et al., 2012). Although many formulations have been developed to increase the bioavailability of curcuminoids, they are often produced for pharmaceutical and food supplement industry, using excipients unsuitable for food industry.

In this work, the *in vitro* bioaccessibility and the degradation of curcuminoids along the gastro-intestinal tract using the *in vitro* gut simulator SHIME® were investigated. A microencapsulated food ingredient, obtained by spray drying the turmeric oleoresin with gum arabic and maltodextrins (GA/MD)[1], was developed and compared with two commercial ingredients, turmeric powder and Meriva®. Moreover, to study the matrix effect, the degradation of curcuminoids was evaluated in two model foods, plain yogurt (a protein-rich food) and rice (a carbohydrate-rich food) enriched with the curcumin-based ingredients.

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[1] Abbreviations: GA/MD, turmeric oleoresin with gum arabic and maltodextrins; GA, gum arabic; maltodextrins, MD; yogurt-GA/MD, plain yogurt enriched with GA/MD; rice-GA/MD, rice enriched with GA/MD; rice-turmeric, rice enriched with turmeric; FE-SEM, Field Emission Scanning Electron Microscope.

2 MATERIAL AND METHODS

2.1 Materials

The turmeric oleoresin was kindly provided by Fiorio colori SpA (Milan, Italy), while the turmeric powder by MB Med srl (Turin, Italy). Meriva® was provided by Indena SpA (Milan, Italy). Meriva® was obtained by mixing curcumin, soy lecithin and microcrystalline cellulose in a 1:2:2 weight ratio, through the Phytosome® strategy, to improve the curcumin bioavailability. The plain yogurt and the rice variety "carnaroli" were purchased at the supermarket. Ethanol, formic acid, acetonitrile, gum arabic (GA), and all analytical standards (curcumin, demethoxycurcumin and bisdemethoxycurcumin) were purchased from Sigma Aldrich (St. Louis, MO, USA), while maltodextrins (MD) (dextrose equivalent 16-20%) was obtained from A.C.E.F. (Fiorenzuola d'Arda, Italy). The enzymes pepsin (3600 U/mg) from porcine gastric mucosa, pancreatin (8 × USP) from porcine pancreas and porcine bile extract were purchased from Sigma-Aldrich (Milan, Italy).

2.2 Microencapsulation of oleoresin by spray drying

Turmeric oleoresin was microencapsulated with *food grade* wall materials in a 95:5 (w/w) ratio of GA and MD mixture and oleoresin, to obtain the microencapsulated ingredient (GA/MD). This microencapsulation was done to improve the water-solubility, stability and bioaccessibility of turmeric oleoresin.

The GA and MD mixture, in a 80:20 (w/w) ratio, was dispersed in water at 60 °C, with magnetic stirring until complete hydration, to a final concentration of 20% (w/v), and then kept in fridge overnight.

Turmeric oleoresin was solubilized in ethanol at a concentration of 2% (w/v) and kept in constant agitation for 1 h at room temperature in the dark. Once solubilized, it was added, dropwise, to the dispersion of wall materials, in a constant stirring at 1000 rpm for 10 min, then mixed using a rotor-stator homogenizer Ultra-Turrax® T25 Basic (Ika-Werke GmbH, Staufen, Germany) at 13500 rpm for 2 min and finally sonicated for 10 min in an ultrasonic bath to obtain a fine emulsion (Sari et al., 2015).

The spray-drying of the emulsion was obtained using the Mini Spray Dryer B-290 *(*Büchi®, Switzerland) and was performed at inlet and outlet temperature of 130 °C and 80 °C, with a feed flow of 7 mL/min and airflow of 40 m^3/h.

The encapsulation yield, calculated as the ratio percentage between the weight of microencapsulated powder and the theorical weight of powder (turmeric oleoresin, GA and MD), was 65%.

2.3 Water Solubility Index (WSI)

The WSI of curcuminoids in oleoresin, GA/MD, Meriva® and turmeric was determined modifying the method described by Kha, Nguyen, & Roach (2010). Saturated solutions of the ingredients were vigorously kept in stirring for 24 h at room temperature and then filtered with 0.45 µm filters (Millipore, Bedford, MA). The filtered solutions were diluted with ethanol and analyzed by HPLC.

2.4 Field Emission Scanning Electron Microscope

The analysis and the morphological characterization of the powdered material produced in this study was performed using a high-resolution system of Scanning Electron Microscopy. Regarding the metallization, the samples were mounted on aluminium stubs with double-sided carbon sticky-tape, sputtered with chrome in a vacuum evaporator and visualized using a ZEISS-SUPRA 25 Field Emission Scanning Electron Microscope (FE-SEM).

2.5 Formulation of foods enriched with curcuminoid-rich ingredients

The ingredients were added to two model foods, plain yogurt and rice, at a curcuminoid concentration of 0.05% (w/w).

The plain yogurt was enriched with only GA/MD (yogurt-GA/MD), because the other two ingredients (Meriva® and turmeric) did not dissolve in this medium. To obtain a homogeneous sample, the yogurt was kept in magnetic stirring, and when the ingredient was dissolved, the sample was treated with the rotor-stator homogenizer at 13500 rpm for 2 min. An aliquot of plain yogurt was used as control.

The rice was enriched with GA/MD (rice-GA/MD) and turmeric (rice-turmeric) to compare a commercial food ingredient to the microencapsulated one. One hundred grams of rice were cooked in 600 mL of boiling water for 14 min. The initial volume of water was determined prior to the experiment as to guarantee that at the end of cooking all the water was absorbed by the rice. The cooked rice was divided in three parts: one was added with the ingredient GA/MD, one with turmeric and one represented the control (white rice). These rice samples were then roughly grinded to simulate the human mastication.

2.6 *In vitro* digestion

To evaluate the *in vitro* bioaccessibility of curcuminoids, the standard INFOGEST protocol (Minekus et al., 2014) was applied to ten grams of food (yogurt-GA/MD, rice-GA/MD and rice-turmeric), 500 mg of GA/MD, 250 mg of turmeric and 25 mg of Meriva®, corresponding to 5 mg of curcuminoids. Salivary amylase (75 U/mL), pepsin (2000 U/mL), pancreatin (corresponding to a trypsin activity of 100 U/mL) with bile salt (10 mM) were used to simulate oral, gastric and intestinal digestion. Samples were digested in triplicate. At the end of each digestive step, one milliliter of digested sample was collected, and the same fractions (stomach and small intestine) of three separate digestions were combined and mixed. The analyses were performed on the combined samples.

2.6.1 Determination of in vitro bioaccessibility

Concerning the intestinal digestion, an aliquot of the sample was further centrifuged at 2300 *x g* for 5 min at 4 °C to separate the soluble (supernatant) and insoluble (pellet) fraction in order to quantify their individual curcuminoid content. The curcuminoid content was determined following protocols described at sections 2.8.4 and 2.9.

The bioaccessibility index of curcuminoids at small intestinal phase was calculated as follows (Ortega, Reguant, Romero, Macia, & Motilva, 2009):

$$\frac{\text{Total curcuminoids in soluble fraction}}{\text{Total curcuminoids in digested sample (soluble + insoluble fractions)}} \text{ x } 100$$

2.7 Twin-SHIME®

The Simulator of Human Intestinal Microbial Ecosystem (SHIME®) was used to simulate the three tracts of colon: ascending, transverse and descending using the settings previously described (Possemiers, Verthé, Uyttendaele, & Verstraete, 2004).

It was set up using two vessels for each tract (Twin-SHIME®) and all vessels were inoculated with the same faecal sample of a healthy human volunteer and stabilized over 2 weeks as previously described (Koper et al., 2018). Inoculum was prepared from freshly voided faecal samples diluted 10-fold in 0.1 M phosphate pH = 7.0 with 1 g/L sodium thioglycolate and centrifuged briefly to remove particulates. SHIME® was fed three times a day with 200 mL of a solution composed of: 1 g/L arabinogalactan, 2 g/L pectin, 1 g/L xylan, 3 g/L potato starch, 0.4 g/L glucose, 3 g/L yeast extract, 1 g/L pepton, 4 g/L mucin and 0.5 g/L cysteine. The pH of the feed was set to 2 and the feed was stored at 4 °C before incubation in each vessel.

The parameters of Twin-SHIME® vessels were: pH 5.6-5.9, volume 500 mL for the ascending vessel; pH 6.1–6.4, volume 800 mL for the trasverse vessel; pH 6.6–6.9, volume 600 mL for descending vessel. The system was kept at 37 °C and in anaerobic conditions by flushing it daily with N_2.

In order to evaluate the curcuminoids degradation by the colonic bacteria, single ingredients and undigested insoluble fractions of rice collected after *in vitro* digestion (see paragraph 2.6.1) were added in a right amount to have the same curcuminoid concentration (5 µg/mL) in each vessel. To allow the transfer in the vessels, samples were firstly hydrated with 2 mL of water. To evaluate the microbial biotransformation over time (5 h), 5 mL of each vessel content were collected at 0, 10, 25, 50, 75, 100, 150, 225 and 300 min, for a total of 9 aliquots. Samples were stored at −20 °C until the analysis.

2.8 Extraction of curcuminoids

2.8.1 Extraction of curcuminoids from the ingredients

Fifty milligrams of GA/MD ingredient were firstly hydrated with 400 µL of water by vortex, extracted with 1600 µL of ethanol, sonicated for 10 min and centrifuged at 20800 *x g* for 10 min at 4 °C. The supernatant was conveniently diluted and analyzed.

One milligram of turmeric was extracted with 1 mL of ethanol/water 80:20 (v:v), sonicated for 5 min and centrifuged at 20800 *x g* for 10 min. The pellet was further extracted with 1 mL of ethanol, sonicated for 5 min and centrifuged. The two supernatants were combined, diluted and analyzed.

One milligram of Meriva® was extracted with 1 mL of ethanol/water 80:20 (v:v), sonicated for 5 min and centrifuged at 20800 *x g* for 10 min. The supernatant was diluted and analyzed.

2.8.2 Extraction of curcuminoids from the yogurt

Five hundred milligrams of yogurt were extracted firstly with 1.5 mL ethanol, mixed by vortex for 1 min, sonicated for 5 min and then centrifuged at 20800 *x g* for 5 min. 1.5 mL of ethanol/water (80:20 v/v) were further added to the residual pellet, sonicated for 5 min and subsequently centrifuged. This step was performed twice and, finally, the three supernatants were combined, diluted and analyzed.

2.8.3 Extraction of curcuminoids from the cooked rice

One hundred milligrams of cooked rice were extracted firstly with 1.5 mL ethanol/water (80:20 v/v) in agitation for 5 min and then centrifuged at 2300 *x g* for 5 min. The supernatant was removed. Afterward, 3 mL of ethanol/water (80:20 v/v) were added to the residual pellet, sonicated for 5 min and subsequently centrifuged. The two supernatants were collected, centrifuged at 20800 *x g* for 5 min, diluted conveniently and analyzed.

2.8.4 Extraction of curcuminoids from digested samples

The curcuminoids were extracted from the entire digested samples (i.e., foods and ingredients) at the end of each digestive step (stomach and small intestine) in order to evaluate the eventual degradation of curcuminoids following the digestion phases. In the case of the pancreatic digestion, digested samples were further centrifuged to separate soluble and insoluble fractions, thus permitting to determine the *in vitro* bioaccessibility (see section 2.6.1).

One hundred milligrams of entire digested sample were extracted with 2 mL of ethanol, mixed for 5 min and centrifuged at 20800 *x g* for 5 min. The supernatant was diluted and analyzed.

The soluble fraction after pancreatic digestion (500 µL) was treated with 0.5 mL of ethanol and mixed for 5 min. The sample was then centrifuged at 20800 *x g* for 5 min and then analyzed.

The insoluble fraction after pancreatic digestion was extracted by adding 3 mL of ethanol to 0.1 g of pellet, mixed for 5 min and centrifuged at 2300 *x g* for 5 min. After that, the pellet was extracted again with 2 mL of ethanol in an ultrasonic bath for 10 min, then centrifuged at 20800 *x g* for 5 min. The two supernatants were combined before the analysis.

2.8.5 Extraction of curcuminoids after colonic fermentation

The extraction of curcuminoids from 0.5 mL of sample incubated in the Twin-SHIME system was preceded by a step of centrifugation at 20800 *x g* for 5 min at 4 °C. Subsequently, the supernatant was eliminated, and the pellet was extracted with 0.5 mL of ethanol, vortexed and sonicated for 10 min. The sample was then centrifuged at 20800 *x g* for 5 min at 4 °C and the supernatant analyzed.

2.9 HPLC analysis

The determination of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) was performed by the method described by Tan et al. (2015) slightly modified. The apparatus was a Thermo Finnigan Surveyor HPLC System (Thermo Scientific, MA, USA) equipped with a photodiode array (PDA) detector. Separation was performed on a reversed-phase Xbridge Shield 18 column (100 mm x 2.1, 3.5 µm; Waters, Massachusetts, USA). The eluents were: (A) acidified acetonitrile (0.1% formic acid, v/v) and (B) acidified water (0.1% formic acid, v/v). The gradient program was: 0-5 min, 95-65 % B; 5-30 min, 65-50 % B; 30-31 min, 50-0 % B; 31-35 min, 0% B; 35-45 min, 0-95%; 45-51 min, 95% B. The flow rate of mobile phase was 0.5 mL/min and the column temperature of 30 °C. The injection volume was 5 µL. Chromatograms were recorded at 425 nm. All compounds were identified comparing retention time and UV/Vis spectra of each respective standard. Calibration curves of each compound, at six different concentration levels, were used for the quantification. Validation parameters of calibration curves are reported as Supplementary Material (Table S1). Results were expressed as total curcuminoids, calculated as sum of the three curcuminoids.

2.10 Statistical analysis

Results were reported as mean ± standard deviation of at least three experiments, excepting for SHIME experiments, for which the analyses were performed in duplicate. The significance of differences was determined by ANOVA, followed by Tukey’s post hoc test ($p < 0.05$) using XLStat 365 (Addinsoft, Paris, France).

3 RESULTS AND DISCUSSION

3.1 HPLC characterization of ingredients and oleoresin

Turmeric oleoresin, the microencapsulated ingredient (GA/MD) and the two commercial products (turmeric and Meriva®) were characterized for their curcuminoids content by HPLC-PDA (**Table 1**). The microencapsulated ingredient and the oleoresin showed a similar composition: 65% of curcumin, 17% of demethoxycurcumin and 18% of bisdemethoxycurcumin. However, the concentration of total curcuminoids in the microencapsulated ingredient (1% w/w) was 24-fold less than that of oleoresin, and half of that in turmeric powder, which had a slightly different composition (71%, 16% and 12% of curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively). This result is in accord with many previous observations, reporting a significant variability in terms of total content of curcuminoids in different sources (turmeric powder, oleoresins, concentrated products) as well as in terms of composition (relative percentage of curcuminoids). Moreover, the curcuminoids content of the raw material (*Curcuma longa* botanical) as well as the environmental parameters can affect the expression and accumulation of bioactive substances (both regarding total volatiles and total non-volatiles compounds) in turmeric (Souza & Glória, 1998). The curcuminoids content of GA/MD was partially affected by the microencapsulation process through spray-drying. In particular, we determined that the encapsulation efficiency of GA/MD, calculated as the percentage ratio between the curcuminoids content determined by HPLC and their theoretic content, was 83%. In a previous work, the encapsulation efficiency of curcumin in microcapsules obtained by spray-drying using a similar blend of coating agent (GA/MD 80:20 supplemented with 1% pullulan) was 65% (Kshirsagar et al., 2009).

The WSI of the turmeric-based ingredients was measured as total curcuminoids concentration in a saturated aqueous solution. The microencapsulated ingredient showed the highest WSI (82 µg/mL), resulting in concentration about 40-fold higher than Meriva® and turmeric (2.1 and 1.8 µg/mL, respectively). Oleoresin was insolubile and no curcuminoids were detected in its aqueous solution. These results are in agreement with our expectations; in fact, GA/MD was prepared using hydrophilic polymers in order to increase the water solubility of curcuminoids. On the other hand, Meriva® is formulated in a phospholipidic matrix (soy lecithin), which improves the *in vivo* bioavailability of curcuminoids, but it is not soluble in water. Therefore, the very low WSI of Meriva® was also expected.

3.2 Morphological analysis of the microencapsulated ingredient

The powdered ingredient GA/MD was characterized in the surface/inner morphology and size of microspheres. FE-SEM analysis showed differently sized microspheres often aggregated in agglomerates, a phenomenon probably also due to the hygroscopic nature of wall material (**Figure 1**). The dimensions ranged from 1 to 15 µm. The smallest microspheres had a dented and collapsed surface, attributable to the partial shrinkage of the particles during the drying process, while the biggest ones showed breakages on the surface and seemed to be a collection of more microspheres. In a previous work, Cano-Higuita, Malacrida, and Telis (2015) observed that microcapsuled particles prepared with GA (alone or associated with MD and modified starch) had generally smooth and continuous surfaces, but in some cases presented dent formation, indicating shrinkage. Collapsed particles are probable related to empty particles, without any inclusion of oleoresins dissolved in ethanol during preparation. Moreover, looking at the morphology of the microspheres, as well as the inner structure visible across the smooth thin transparent surface, a porous structure can be easily highlighted. The porous matrix can be generated by the evaporation of the ethanol at high temperature during the spray-drying process. As generally accepted, this typology of matrix can improve significantly the contact with the aqueous solvent, which depends on the increase of the total area of the surface, leading the improvement of the hydrophilic properties, as highlighted during the bioaccessibility tests (see below). Indeed, porous microspheres had interconnective external and internal pores leading to very low mass density and enormous specific surface area, enabling them to have excellent adsorption capabilities (Dastidar, Saha & Chowdhury, 2018). Moreover, this porous system can dramatically improve the dissolution rates of biomolecules in hydrophilic medium, allowing the microspheres work as functional microcarrier for the *in vivo* delivery of bioactive compounds (Ebrahimi, Saffari & Langrish, 2017).

Other studies on curcumin encapsulation confirmed the possibility to obtain also nanoparticles with interesting properties (e.g. enhanced antimicrobial properties), reaching a narrow particle size distribution in the range of 2-40 nm. The nanodimension of these materials can strongly improve their dispersibility in water, as observed in the case of microspheres prepared in this work. The enhanced antimicrobial properties of these nanoparticles containing curcumin when compared to raw oleoresin is explained by their capacity to be integrated in the microbial cell of GRAM+ and GRAM- bacterial strains. This effect must be deeply studied *in vivo*, considering the chance to interact with the gut microbiota (Basniwal, Buttar, Jain & Jain, 2011).

3.3 Degradation of curcuminoids in curcuminoid-rich ingredients along the gastro-intestinal tract

GA/MD, turmeric and Meriva® underwent an *in vitro* digestion to evaluate the curcuminoids degradation, in different formulations, at physiological conditions (pH, temperature and enzymes). The total curcuminoids of each formulation before digestion (undigested ingredient), and after the gastric (pepsin) and the intestinal phase (pancreatin) are displayed in **Table 2**. A significant decrease in the amount of curcuminoids in all the ingredients was measured at the end of the two digestion phases. The highest decrease was observed during intestinal phase in GA/MD (-32%), whereas a similar and lower decrease was measured for turmeric and Meriva® (-16% and -17%, respectively). GA/MD had the highest loss already in the gastric phase (-20%), when compared to turmeric (-14%) and Meriva® (-9%). Similar results were observed by Aniesrani Delfiya, Thangavel, Natarajan, Kasthuri, and Kailappan (2015), who highlighted that the curcuminoids microencapsulated with GA were released more quickly in an acid medium than those present in oleoresin because of the fast hydrolysis of curcumin-GA bond. Meriva® was the formulation that showed the highest stability to the gastro-intestinal conditions. This was likely due to the presence of phospholipids that protect the sensitive active agents from the degradation (Fricker et al., 2010). Based on all these observations, we can hypothesize that the high curcuminoids degradation in GA/MD following digestion is to be related to the hydrophilic properties of coating agents, which have also contributed to the the highest WSI value of GA/MD (**Table 1**).

To determinate the bioaccessible fraction of total curcuminoids, the digested samples were separated by centrifugation in two fractions, soluble (bioaccessible) and insoluble (non-bioaccessible). **Figure 2** shows the percentage of soluble and insoluble curcuminoids in GA/MD, Meriva® and turmeric, expressed with respect to their total content after digestion. The obtained values highlight a higher bioaccessibility in the ingredients GA/MD (4.9 ± 0.7) and Meriva® (5.7 ± 0.5), in which the curcuminoids are embedded into a lipophilic phospholipid environment able to form micro-emulsion (Ahmed, Li, McClements, & Xiao, 2012; Zou et al., 2015), compared to the turmeric powder (0.18 ± 0.05). It is also interesting to observe that, despite their dramatically different WSI, GA/MD and Meriva® present very similar bioaccessibility. Gum arabic and maltodextrins are polymers characterized by high-water solubility that leads to the dissolution of curcuminoids in the simulated gastro-intestinal fluids. Instead, Meriva® is formulated with phospholipids (soy lecithin), which have very low water solubility, but can be digested by pancreatic lipases, thus releasing curcuminoids in the digestion medium. For this reason, they have similar bioaccessibility.

The degradation of curcuminoids in each section of the colon was investigated by incubating the three ingredients with the microbiota present in the three different SHIME® compartment. The initial concentration of total curcuminoids for each sample was the same in each vessel (5 µg/mL). The degradation of total curcuminoids in each colon section is showed in **Figure 3**.

To the best knowledge of the authors, this is the first time the degradation of curcuminoids was studied in each single section of a human colon simulator. After 5 h of incubation, the loss of curcuminoids was about one third in the ascending colon (29% for Meriva®, 33% for GA/MD and 37% for turmeric). In the transverse colon, the loss ranged from 34% in Meriva® to 60% in turmeric. In the descending colon, there was the highest loss ranging from 46% (Meriva®) to 61% (turmeric). Overall, Meriva® displayed the highest stability to microbial biotransformation in each colon section when compared to the other ingredients, followed by GA/MD and turmeric. The higher degradation of curcuminoids in the last section of colon was probably due to the higher pH of this colon section with respect to the other ones.

3.4 Evaluation of food matrix effect on the degradation of curcuminoids along gastro-intestinal tract

To evaluate the food matrix effect on the degradation of curcuminoids along the gastro-intestinal tract, the yogurt and the rice enriched with curcuminoid-rich ingredients (rice-GA/MD, rice-turmeric and yogurt-GA/MD) were underwent the same protocol of simulated digestion. The results showed in **Table 3** highlight that the gastric degradation of curcuminoids in the yogurt (-10%) is lower if compared to that occurring in the rice (-17% for rice-GA/MD and -19% for rice-turmeric), but these differences disappeared after the intestinal digestion.

The results of bioaccessibility (**Fig. 4**) show a content of curcuminoids higher in the soluble fraction of rice samples (6% and 9% for rice-turmeric and rice-GA/MD, respectively) compared to yogurt (1.3%), with a bioaccessibility about 7 times higher for rice-GA/MD when compared to yogurt-GA/MD.

Interestingly, the solubility of curcuminoids increased when the ingredients were added to the rice compared to when they were digested alone; particularly, the bioaccessibility increased almost 2 times for GA/MD, and 30 times for turmeric. These results demonstrated the positive effect of the presence of rice matrix on the bioaccessibility of curcuminoids. A similar result was observed in a clinical trial by Vitaglione et al. (2012). The authors demonstrated that the curcuminoids encapsulated with cellulose derivates and vegetable oil and provided in a carbohydrate-based food (i.e. bread) were more bioavailable compared to the non-encapsulated ones.

The bioaccessible fraction of yogurt-GA/MD was instead just the 1.3%. This result was unexpected since it has been demonstrated that the presence of emulsified lipids (like fats in a plain yoghurt) increases the bioaccessibility of curcumin with respect to the presence of non-emulsified ones (Zou et al., 2015). Probably, the curcuminoids had a major affinity with yogurt lipids that favored the passage of curcuminoids in the insoluble fraction during the step of centrifugation, while during the *in vivo* digestion they are entrapped in micelles that increased their bioaccessibility and absorption (Fu, Augustin, Sanguansri, Shen, Ng & Ajlouni, 2016).

The GA/MD enriched rice was also tested in the SHIME® system. The rice-turmeric was used as the control since we would investigate whether the new ingredient (i.e., GA/MD) had a different behaviour with respect to the traditional one (i.e., curcumin). After the gastro-intestinal digestion of rice-GA/MD and rice-turmeric, their insoluble fractions underwent 5 h colonic fermentation in the ascending, transverse and descending colon section (**Fig. 5**). As previously observed for the ingredients alone, the curcuminoid degradation was lower for the enriched rice in the ascending colon than in the other two tracts. The percentage of decrease was 34%, 44% and 49% for rice-GA/MD, and 26%, 52% and 55% for rice-turmeric in ascending, transverse and descending colon, respectively.

Overall, even if the curcuminoids degradation by the colonic bacteria appeared slightly higher when the ingredients were alone compared to when they were added to rice, no statistically significant differences were found.

4 Conclusions

Low bioaccessibility and stability of curcuminoids limit the beneficial properties of turmeric. In this study, we investigated the effect of a microencapsulation with food grade wall materials of turmeric oleoresin on the *in vitro* bioaccessibility and microbial biotransformation of curcuminoids, and compared them to the non-encapsulated commercial ingredient (turmeric) and to a patented ingredient for pharmaceutical uses (Meriva®).

The curcuminoids were significantly degraded from the gastro-intestinal conditions in all formulations. However, the curcuminoid *in vitro* bioaccessibility in the microencapsulated ingredient (i.e. GA/MD) resulted 25-fold higher than that of turmeric powder. The porous microspheres are described as promising carriers for bioactive compounds, particularly regarding aqueous food systems. Moreover, the curcuminoid bioaccessibility of rice enriched with the spray-dried ingredient was about 2- and 1.5- fold higher with respect to that of the ingredient alone and of Meriva®, respectively. Unexpectedly, curcuminoids were more bioaccessible in rice than in yoghurt and the rice matrix protected the curcuminoids from their bacterial degradation that occurs during the transit in the three tracts of a human colon simulator.

In future, further studies will be needed to evaluate the performances of the formulated microencapsulated ingredient in other food matrices, like those containing phospholipids (eggs or vegetable oils). Moreover, the contribution of other food grade wall materials to the gastro-intestinal stability of the main compounds should be studied as well. Finally, it is worth to notice the use of these nanomaterials in Europe is currently restricted, and this is a severe limitation to the exploitation of this kind of the curcumin-containing ingredients.

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Table 1 Curcuminoids in oleoresin and in curcuminoid-rich ingredients. Values are expressed as mg of curcumin, demethoxycurcumin and bisdemethoxycurcumin per gram of sample. The relative percentage of each curcuminoid on the total curcuminoids is reported in the bracket. The total curcuminoids content (%, w/w in respect to the weight of sample) and the WSI of curcuminoids in each ingredient (expressed as total curcuminoid concentration in a saturated aqueous solution, µg/mL) are also reported.

	Curcumin (mg/g)	**Demethoxy-Curcumin (mg/g)**	**Bisdemethoxy-Curcumin (mg/g)**	*Total curcuminoids content (%)*	*WSI µg/mL*
Oleoresin	160 ± 7 (66%)	41.2 ± 0.8 (17%)	39.6 ± 1.1 (17%)	*24*	-
GA/MD	6.20 ± 0.26 (63%)	1.75 ± 0.04 (18%)	1.89 ± 0.02 (19%)	*1*	*82*
Turmeric	14.4 ± 0.3 (71%)	3.26 ± 0.05 (16%)	2.49 ± 0.05 (12%)	*2*	*1.8*
Meriva®	168 ± 6 (82%)	27.3 ± 1.8 (13%)	8.69 ± 0.04 (5%)	*20*	*2.1*

Table 2 Effect of the *in vitro* gastro-intestinal digestion on curcuminoid content of curcuminoid-rich ingredients Values are expressed as mg of total curcuminoids per gram of digested ingredient. Percentage of degradation of the digested sample compared to the undigested one is reported in the bracket. Different letters represent significant differences within the same sample (column) ($p<0.05$).

	GA/MD (mg/g)	**Turmeric (mg/g)**	**Meriva® (mg/g)**
Undigested	10.3±0.8^{a}	20.1±0.4^{a}	204±8^{a}
Gastric phase	8.22±1.13^{b} (-20%)	17.3±1.3^{ab} (-14%)	186±15^{ab} (-9%)
Intestinal phase	7.05±0.77^{b} (-32%)	16.8±1.2^{b} (-16%)	169±7^{b} (-17%)

Table 3 Effect of an *in vitro* gastro-intestinal digestion on curcuminoid content in foods. Values are expressed as µg of total curcuminoids per gram of digested food. Percentage of variations of the digested sample compared to the undigested one is reported in the bracket. Different letters represent significant differences within the same sample (column) ($p<0.05$).

	Rice-GA/MD (µg/g)	**Rice-turmeric (µg/g)**	**Yogurt-GA/MD (µg/g)**
Undigested sample	418±27 ^{a}	426±64 ^{a}	478±17 ^{a}
Gastric phase	347±8 ^{ab} *(-17%)*	344±26 ^{ab} *(-19%)*	429±29 ^{a} *(-10%)*
Intestinal phase	304±6 ^{b} (-27%)	289±25 ^{b} *(-32%)*	358±41^{b} *(-25%)*

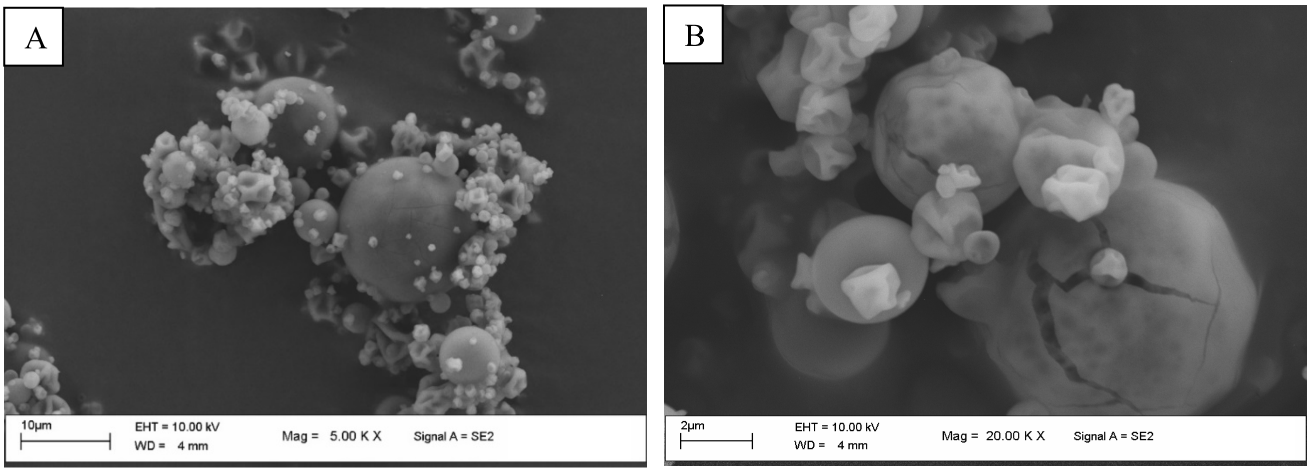
Figure 1. Fe-SEM image of the microencapsulated ingredient (GA/MD). Panel A) magnification: 5000x. Panel B) magnification: 20000x.

Figure 2. Bioaccessibility of curcuminoids after the *in vitro* gastro-intestinal digestion of the curcuminoid-rich ingredients. Values are expressed as percentage of soluble (bioaccessible) and insoluble (non-bioaccessible) curcuminoids in respect to the total content in digested sample.

Figure 3. Effect of 5-h microbial biotransformation on the curcuminoid content of the curcuminoid-rich ingredients. Data are expressed as the percentage of variation of total curcuminoids at t_{300} compared to those at t_0 in ascending, transverse and descending colon. For each colon tract, values accompanied by the same letter are not significantly different.

Figure 4. Bioaccessibility of curcuminoids in foods after the *in vitro* gastro-intestinal digestion. Values are expressed as percentage of soluble (bio-accessible) and insoluble (non-bioaccessible) fraction in respect to the total content of digested food.

Figure 5. Effect of 5-h microbial biotransformation on curcuminoid content in rice. Data are expressed as the percentage of variation of total curcuminoids at t_{300} compared to those at t_0 in ascending, transverse and descending colon. For each intestinal tract, values accompanied by the same letter are not significantly different.


A
10µm
EHT = 10.00 kV
WD = 4 mm
Mag = 5.00 K X
Signal A = SE2
B
2µm
EHT = 10.00 kV
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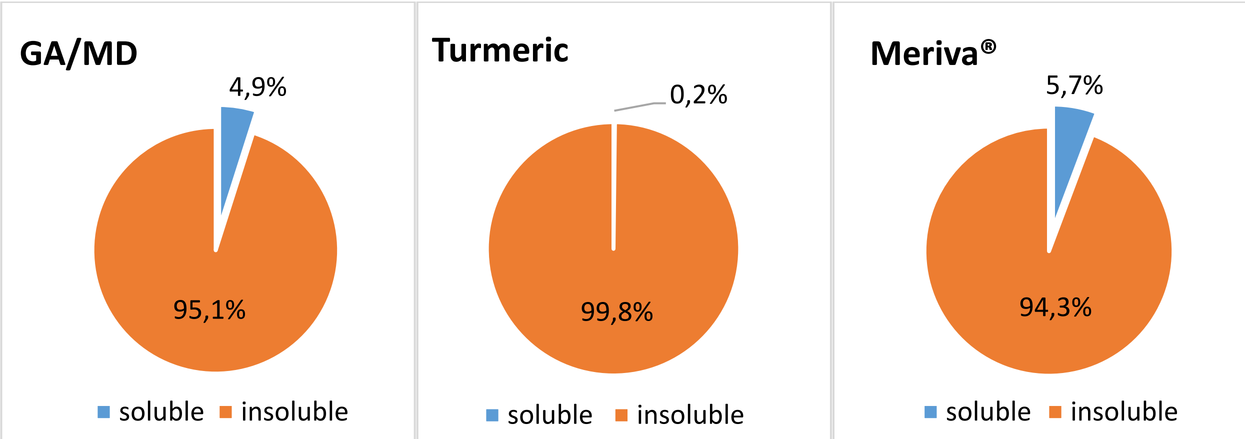
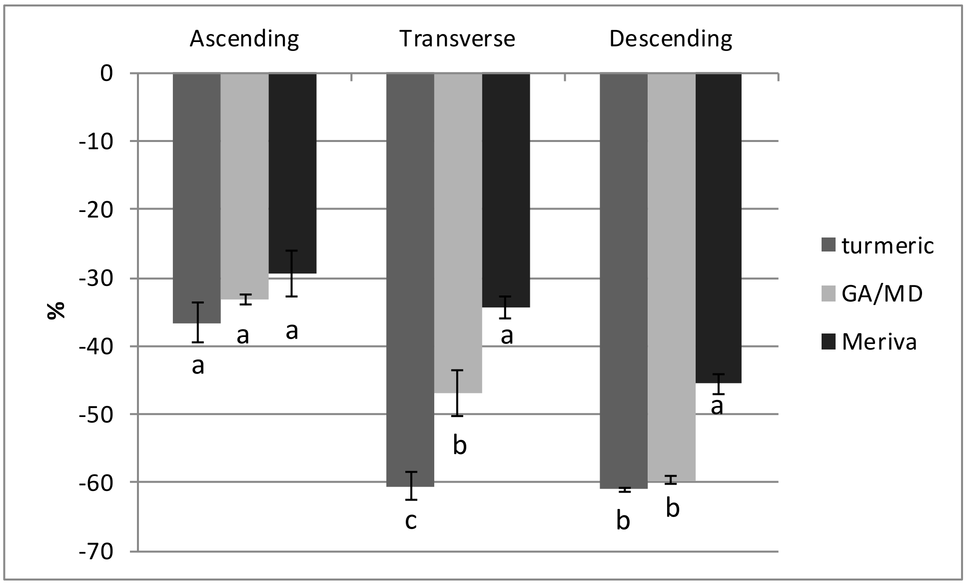
Mag = 20.00 K X
Signal A = SE2


GA/MD
4,9%
95,1%
soluble
insoluble
Turmeric
0,2%
99,8%
soluble
insoluble
Meriva®
5,7%
94,3%
soluble
insoluble


Ascending
Transverse
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0
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-20
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%
turmeric
GA/MD
Meriva
a
a
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c
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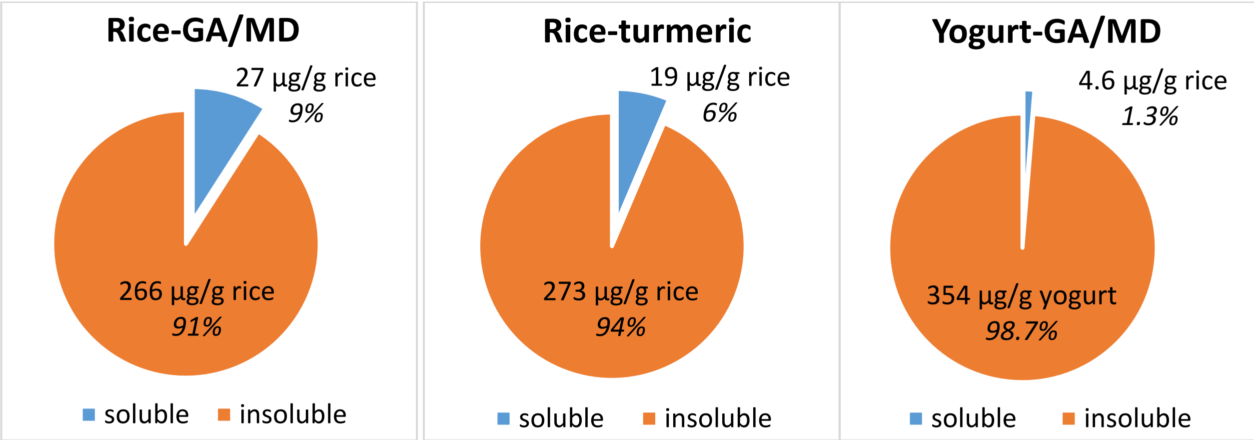
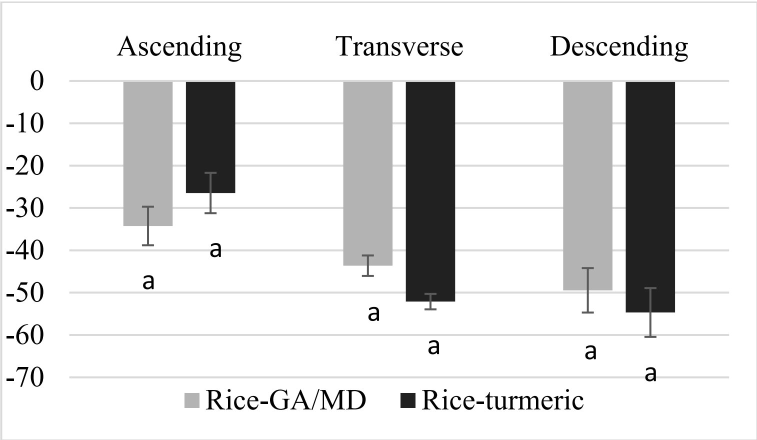
a
b
b
a


Rice-GA/MD
27 µg/g rice
9%
266 µg/g rice
91%
soluble
insoluble
Rice-turmeric
19 µg/g rice
6%
273 µg/g rice
94%
soluble
insoluble
Yogurt-GA/MD
4.6 µg/g rice
1.3%
354 µg/g yogurt
98.7%
soluble
insoluble


Ascending
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0
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Rice-GA/MD
Rice-turmeric