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BIOACCESSIBILITY OF LYCOPENE: THE CRITICAL ROLE OF DIETARY FIBER INTERACTIONS IN DEVELOPMENT OF FOOD FOR SPECIAL MEDICAL PURPOSES

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Summary

Lycopene is gaining recognition as a functional food component due to antioxidant and immunomodulatory properties that result with numerous health benefits. However, the interaction between lycopene and the food matrix, especially dietary fiber (DF), significantly impacts its bioaccessibility from food. This study investigates how different types of DF influence lycopene bioaccessibility, which is crucial for developing effective functional foods. The research utilized DF of different characteristics (inulin, oligofructose, dextrin, gummi arabicum, cellulose, pea fiber, apple fiber, and citrus fiber) to assess their lycopene binding capacity (LBC). Obtained results were further used to investigate if single fiber – LBC data can be used to predict LBC of DF mixture (by comparing experimental and calculated data). The study involved *in vitro* static simulation of gastrointestinal digestion to mimic gastric and intestinal conditions. The content and characteristics of DF were assessed by standard AOAC methods. Lycopene content was measured spectrophotometrically. Results showed that lycopene itself is stable under gastrointestinal conditions. However, the presence of different DF significantly altered lycopene bioaccessibility, that ranged from 50.7% to 111.3%, depending on the fiber type. The content of insoluble DF, higher oil holding capacity and shorter DF chain length corresponded with a lower lycopene bioaccessibility. Data on LBC of DF enabled relatively accurate prediction of LBC of DF mixtures. Understanding DF−lycopene interactions is crucial for formulating functional foods that maximize the health benefits of both lycopene and DFs. Further research should focus on the structural and physicochemical characteristics of DFs to optimize their combination with lycopene in food formulations, enhancing their bioavailability and health benefits.

Keywords: lycopene, dietary fiber, lycopene binding capacity, bioaccessibility, functional food

Introduction

Lycopene, a carotenoid found mainly in tomatoes and other red fruits, has become known for its significant health benefits, particularly in the area of medical foods. Its antioxidant properties are paramount, making it a component of interest in the prevention and treatment of various chronic diseases. Research indicates that lycopene can play a role in reducing the risk of certain cancers, particularly prostate and colorectal cancers, as well as mitigating metabolic disorders such as obesity and type 2 diabetes. Studies have shown that lycopene improves outcomes related to these metabolic diseases by alleviating oxidative stress and inflammation, which are significant contributors to their progression (Shafe et al., 2024; Kulawik et al., 2023). A recent review found that dietary lycopene intake (or serum lycopene levels) was inversely associated with all-cause mortality, prostate cancer, stroke, cardiovascular disease, metabolic syndrome, and male infertility (Li et al., 2021).

As the continuing research continues to uncover the potential health benefits of lycopene, it is increasingly being used in dietary supplements and functional foods to promote health and prevent chronic diseases. The global lycopene market is projected to reach approximately $196.6 million by 2030, growing at a compound annual growth rate (CAGR) of 4.9% from 2023 to 2030, reflecting a rising demand for natural antioxidants in the food and beverage sector. Furthermore, the increasing preference for ″clean label″ products has led manufacturers to favor natural ingredients like lycopene over synthetic alternatives. This shift is particularly evident in North America and Europe, where consumers are actively looking for foods that support health while avoiding artificial additives (Bothare, 2024).

Despite the growing body of evidence for the health benefits of lycopene, there remains a notable gap in understanding how exactly food matrix interacts with this carotenoid and affects its stability and bioaccessibility, and thus its bioavailability (Shahidi and Pan, 2022). This lack of information hinders the development of optimized formulation strategies that could enhance the bioavailability of lycopene from functional/special medical food formulations. Even

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though it is known that dietary fiber (DF), together with proteins, are the components of the food matrix that have the greatest impact on lycopene bioaccessibility (Molteni, 2022, 12.14.; Núñez-Gómez, 2023), the exact mechanisms of DF−lycopene interactions and the importance of particular DF characteristics on the ability to form complexes with lycopene have not been investigated so far. With the increasing need for foods for special medical purposes, which are usually rich in both DF and bioactive compounds such as carotenoids, there is a need to get better insight into structural- or physico-chemical characteristics of DF that are responsible for the occurrence of physiologically important interactions with bioactive compounds, in this case, lycopene. Therefore, the understanding physiologically relevant lycopene−DF interactions and the general acceptance of the bioaccessibility-based approach in functional food development is crucial for the formulation of functional foods that can maximize the health benefits of both DFs and lycopene (Xavier, and Mercadante, 2019). The main goal of this study is to investigate more thoroughly the significance of interactions of lycopene with DF/DF mixtures since it is essential for advancing our understanding and application of lycopene in dietary practices aimed at disease prevention and health promotion.

Materials and methods

DF (inulin, oligofructose, dextrin, gummi arabicum, cellulose, pea fiber, apple fiber and citrus fiber) utilized in this research were donated by Belupo Inc. (Koprivnica, Croatia). DF mixtures investigated for lycopene binding capacity (LBC) were designed by taking into account data on LBC for pure fibers and particular physico-chemical characteristics of pure fibers. The approximate composition of DF mixtures is presented in Table 1.

Table 1. Composition of dietary fiber mixtures

	IN/FOS /DEX+ CEL	**IN/FOS /DEX +CEL[1]**	**MIX**	**MIX+ CEL**	**MIX***	**MIX+ CEL***	**SOL**	**SOL+ CIT**	**SOL+ CIT+ PEA**
	Impact of increasing cellulose content		Impact of adding cellulose to the mixture/impact of the citrus DF particle size				Impact of adding citrus - and/or pea DF in the mixture		
FOS	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	+	+	+
Dextrin	+	+	+	+	+	+	+	+	+
Cellulose II[1]	++	+	-	+	-	+	-	-	-
Gummi arabicum	-	-	+	+	+	+	+	+	+
Citrus fiber I [2]	-	-	+	+	-	-	-	+	+
Citrus fiber II [3]	-	-	-	-	+	+	-	-	-
Pea fiber	-	-	+	+	+	+	-	-	+

[1] fiber size 180 μm; [2]fiber size < 30 μm; [3]fiber size 75 μm. – fiber is not contained in the mixture

Lycopene used in experiments was Redvivo® from DSM (Limburg, Netherlands) – it is the formulation of lycopene uniformly dispersed in the modified starch matrix to ensure adequate water solubility. Total DF Assay Kit was from Megazyme® (Bray, Ireland). Bile salts, pancreatin from porcine pancreas (8 × USP) and lycopene ≥98% (HPLC) from tomato used for the preparation of calibration curve were from Sigma–Aldrich (St. Louis, MO, USA). Pepsin (from porcine gastric mucosa) 0.7 FIP-U/mg was from Merck (Darmstadt, Germany). All other chemicals were from Kemika (Zagreb, Croatia).

The amount of the total-, soluble- and insoluble- DF was determined using the Megazyme® procedure based on AOAC Method 991.43 "Total, Soluble, and Insoluble Dietary Fiber in Foods" *(*McCleary, 2023). It involves preparing an enzymatic digestion of the sample with heat-stable α-amylase at boiling temperature for 30 minutes, cooling the mixture, and treating it with protease at 60 °C for 30 minutes. After adjusting the pH, amyloglucosidase was added and incubated again. The mixture was then filtered to separate insoluble dietary fibre (IDF) from soluble dietary fibre (SDF), with SDF precipitated using ethanol. Finally, both fractions were dried and weighed to calculate the total dietary fibre (DF) content by summing the weights of IDF and SDF.

Water holding capacity (WHC) determination was based on the procedure of Robertson and Eastwood (1981) with some modification. Briefly, 50 mL Falcon tubes were labeled and weighed. 0.5 g of pure DF was placed into each Falcon tube, 20 mL of water was added to the fibers and the tubes were incubated at room temperature overnight. After incubation, the

samples were centrifuged for 10 minutes at 4200 rpm to separate unbound water from the hydrated fibers. Following centrifugation, any remaining free water was carefully removed using an automatic pipette, ensuring that the sediment (hydrated fibers) remains undisturbed. Next, the Falcon tubes containing the hydrated fibers are weighed again to determine their final mass and the mass of bound water. WHC was calculated using formula [1].

$$WHC\ (\frac{g}{g}) = \frac{mass\ of\ bound\ water\ (g)}{mass\ of\ fibre\ (g)} \qquad [1]$$

For the determination of oil holding capacity (OHC) modified procedure of Larrauri and co-workers (1996) was used, with some modification. Briefly, 15 mL Falcon tubes were weighed; 0.5 g of pure DF was added, following by the addition of 5 mL of sunflower oil. The tubes were incubated at 4 °C for 1 h and the samples were centrifuged for 15 minutes at 4200 rpm to separate any unabsorbed oil from the hydrated fibers. Following centrifugation, excess oil was carefully removed and the Falcon tubes containing the bound oil were weighed again to determine their final mass. OHC was calculated using formula [2].

$$OHC\ (\frac{g}{g}) = \frac{mass\ of\ bound\ oil\ (g)}{mass\ of\ fibre\ (g)} \qquad [2]$$

Gastrointestinal stability of lycopene and DF binding capacity were obtained through an *in vitro* static simulation of gastrointestinal digestion, based on the standardized protocol established by Brodkorb et al. (2019), with some modifications. Initially, the samples were incubated in simulated gastric fluid (SGF) containing pepsin to mimic gastric conditions, maintained at 37 °C for 2.5 h in a water bath (Büchi B-490, Flawil, Switzerland) with consistent shaking at 110 rpm. Following this incubation, simulated intestinal fluid (SIF), which included bile salts and pancreatin, was added to the samples, and the reaction mixtures were incubated under identical conditions for another 2.5 h. Afterward, the samples were placed on ice for 10 minutes and then filtered through polypropylene hydrophilic membranes with a pore diameter of 20 µm to obtain clear filtrates suitable for the spectrophotometric determination of lycopene at 503 nm. Obtained absorbances were corrected by subtracting the absorbance of blanks containing SGF, SIF and enzymes. The concentration of lycopene was calculated based on the calibration curve of lycopene according to the equation [3].

$$y = 0.0044\text{x} - 0.0178 \qquad [3]$$

where y is a lycopene concentration (mg/L), and x is the absorbance measured at 503 nm and corrected by the absorbance of blank.

For the preparation of lycopene−DF mixtures lycopene was dissolved in SGF in the concentration of 300 mg/L. 5 mL of this solution was added to 120 mg of pure fibers or 600 mg of the fiber mixtures in a 15 mL Falcon tube. The mixture was vortexed for 60 s to resuspend all the fibers. For the determination of lycopene gastric bioaccessibility (t=0 h) the mixture was centrifuged, and lycopene content was determined in the supernatant. For determination of gastric (t=2.5 h) or intestinal (t=5 h) bioaccessibility simulation of gastric or gastric and intestinal digestion was conducted as described above, prior to determination of lycopene content in the bioaccessible fraction.

In vitro bioaccessibility of lycopene from model mixtures was calculated according to the equation [4].

$$bioacessibility\ (\%) = \frac{bioaccesible\ lycopene}{total\ lycopene}\ x\ 100 \qquad [4]$$

while total lycopene was determined after incubating the solution of lycopene (300 mg/L) in distilled water, in the dark, at room temperature, for 5 h. Theoretical bioaccessibility of lycopene form the fiber mixtures was calculated by multiplying the lycopene bioavailability from the model solution with a single DF with its ratio in the DF mixture and summarizing all obtained values.

Statistical analysis

DF content, WHC, OHC and lycopene bioaccessibility were analyzed in duplicates (n). DF–lycopene mixtures were prepared in duplicates, and each sample was assessed in triplicate for the lycopene content (n=6). Results are presented as means ± standard deviations. Obtained results were compared using Student's t-test or one-way analysis of variance and obtained differences were considered statistically significant when $p<0.05$. Software used for statistical analysis and graphical presentation of obtained results was Microsoft Excell (Microsoft Corporation, Washington, USA) and GraphPad Prism ver. 8.4.3 (GraphPad Software, Inc., CA, USA).

Results and discussion

The physico-chemical properties of food matrix can significantly influence the efficiency of digestion, especially by interfering with the interaction of the particular nutrient with digestive enzymes. The interaction required for successful absorption can only occur if the DF rich cell walls in plant tissues are broken down or the enzymes can diffuse through permeable cell

walls. Since not all plant cell wall matrices behave the same during digestion, the highly permeable or easily disrupted cell walls enable greater bioaccessibility and earlier digestion of nutrients (Van den Bringen, 1999; Nunez-Gomez, 2023). On the other hand, free nutrients can be encapsulated by DF under the conditions of gastrointestinal tract which may hinder their incorporation into micelles necessary for absorption in the intestines but also, in some cases, protect them from the degradation in gastrointestinal tract.

As shown in Figure 1, lycopene used in this study proved to be stable under the conditions of the gastrointestinal system. The amount of bioaccessible lycopene changed from 94.9 ± 0.9 % at the beginning of gastric digestion up to 108.4 ± 8.9 % at the end of intestinal digestion with no significant difference between obtained results ($p>0.05$). This is not consistent with the available research on *in vitro* gastrointestinal stability of lycopene naturally present in food. Bilušić and co-workers (2019) showed significantly lower gastrointestinal stability of lycopene ranging from 59.8 ± 0.2 % (in carrot) up to 68.1 ± 0.1 % (red pepper). Similarly, Goni and co-workers (2006) showed that bioaccessibility of lycopene from vegetables is around 82% and from fruits around 33.3%. The authors assumed that low bioaccessibility is due to the presence of insoluble protein-lycopene and DF−lycopene complexes in the food matrix. Similar negative effect of DF on lycopene bioaccessibility has been confirmed by several scientific investigations *in vivo* (Riedl et al., 1999; van Het Hof, 2000). The main reason for higher bioaccessibility of lycopene in our study is the fact that lycopene naturally present in food exists in carotenoid-protein complexes or forms crystalline aggregates tightly bound to proteins that protect it from degradation but limit its release during digestion. In our study, free lycopene was added to the mixture of DF so its bioaccessibility could be reduced only in case of forming insoluble complexes during simulation of digestion or due to degradation under gastrointestinal conditions.

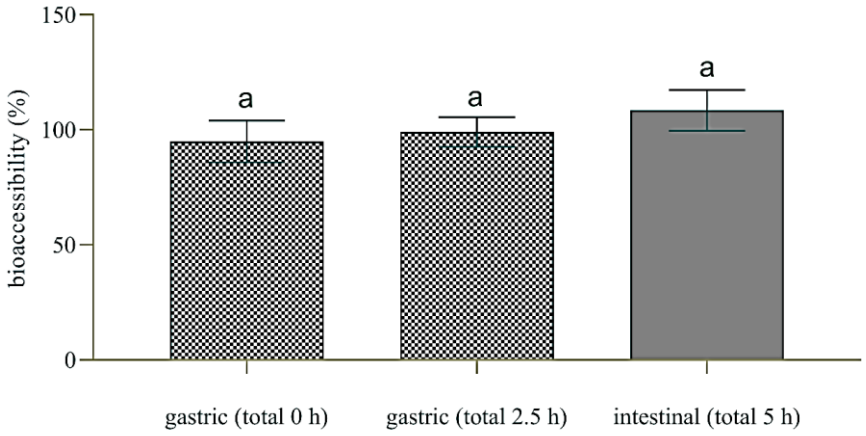

Fig. 1. Gastrointestinal bioaccessibility of lycopene
Columns marked with the same letters belong to the same statistical groups ($p>0.05$) as assessed by ANOVA and post hoc Tukey test.

Major characteristics (OHC, WHC, total-, soluble- and insoluble- DF content) of DF utilized in this investigation are presented in Table 2.

Table 2. Characteristics of pure dietary fiber analyzed for lycopene binding capacity

	OHC (g/g)	WHC (g/g)	TDF (g/100g)	SDF (g/100g)	IDF (g/100g)
fructooligosaccharides	0.8±0.06	-	88.5±4.2[4]	88.5±4.2[5]	-
inulin	1.1±0.02	1.5±0.15	95.2±7.1[4]	95.2±7.1[5]	-
dextrin	1.8±0.03	-	84.5±3.2	84.5±3.2	*
cellulose I[1]	3.1±0.04	5.8±0.47	99.0±6.5	0.66±0.05	98.3±4.3
cellulose II[2]	1.2±0.01	4.5±0.03	98.5±2.1	-	98.5±2.1
gummi arabicum	1.3±0.09	-	90±5.1	88.4±4.5	1.6±0.1
citrus fiber I[3]	1.2±0.09	8.2±0.45	70±4.3	31.9±0.1	38.8±4.1
citrus fiber II[4]	1.2±0.02	9.9±0.22	70±3.5	31.9±0.2	38.1±2.3
peas fiber	1.1±0.01	5.0±0.04	59.3±3.1	6.2±0.07	53.1±6.1
apple fiber	1.3±0.05	4.4±0.12	61.2±4.8	41.5±0.3	19.5±2.2

OHC-oil holding capacity; WHC-water holding capacity; TDF-total dietary fiber; SDF-soluble dietary fiber; IDF-insoluble dietary fiber:[1] fiber size 75 μm; [2] fiber size 180 μm; [3]fiber size < 30 μm; [4]fiber size 75 μm, [5]data obtained from the producer (since fructan-type fiber cannot be determined by the dietary fiber determination method applied)-not determined.

The results presented show that commercially available DF differ significantly regarding their purity i.e., TDF content. Obtained values ranged from 59.3% (in peas fiber) up to 99.0% in cellulose fiber. Four types of analyzed DF were predominantly soluble (FOS, inulin, dextrin and gummi arabicum); two types were predominantly insoluble (two types of cellulose, with different chain lengths) and four types of DF were mixed type, containing both soluble and insoluble fraction, in different ratios (two types of citrus fiber, peas fiber and apple fiber).

Results presented in Table 2 also show that there are significant differences in OHC and particularly WHC among tested DF, as well. WHC and OHC are fundamental characteristics that influence the physiological effects of DF, impacting digestion, nutrient absorption, and overall gut health. WHC allows DFs to retain water, which is essential for increasing stool bulk and promoting regular bowel movements. Limited literature data indicate that DF with high WHC can also trap nutrients, including water-soluble vitamins and minerals, thereby affecting their absorption. The OHC is important for its ability to bind fats and fat-soluble nutrients which might modulate the absorption of liposoluble compounds from food. WHC values ranged from 1.5 ± 0.2 g/g or inulin, up to 9.9 ± 0.2 g/g for citrus fiber II. OHC ranged from 0.8 ± 0.1 g/g in FOS up to 3.1 ± 0.0 for cellulose I. Obtained values are consistent with available literature data (Jiang 2022, Wagner 2024, data obtained from the manufacturer).

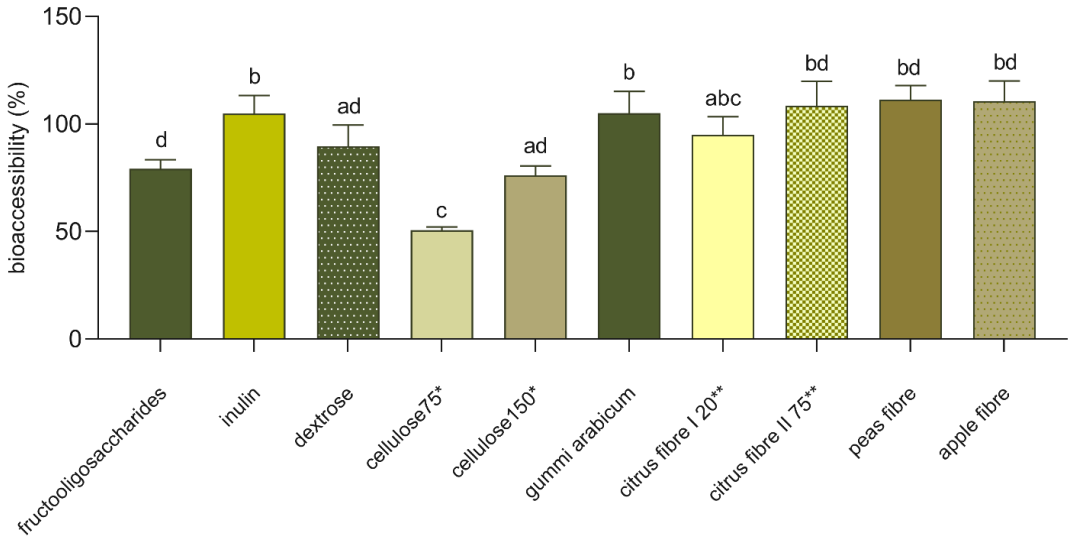

Fig. 2. *In vitro* bioaccessibility of lycopene from model mixtures with dietary fiber
Columns marked with different letters belong to different statistical groups ($p<0.05$). *Approximate length of dietary fiber; **average mesh size. cellulose I-fiber size 75 µm; cellulose II-fiber size 180 µm; citrus fiber I-fiber size < 30 µm; citrus fiber II-fiber size 75 µm.

Results presented in Figure 2 show that investigated DF differ significantly according to their LBC resulting in the wide range of lycopene bioaccessibility. Observed values ranged from 50.7 ± 1.5 % from the model solution containing cellulose I up to 108.4±11.5 % – 111.3±6.6 % in the model solutions containing citrus fiber II and peas fiber, respectively. As presented in Table 3, the amount of TDF in the reaction mixture showed significant negative correlation with lycopene bioaccessibility (r=-0.6849; p=0.0289) and the effect was even more pronounced in the case of IDF (r=-0.8167; p=0.0250). The amount of SDF in the model solution seems to have no influence on the bioaccessibility of lycopene.

Table 3. Correlation between total dietary fiber (A), soluble dietary fiber (B), insoluble dietary fiber (C) and OHC (D) and lycopene bioaccessibility

	Correlation coefficient	**correlation**	**Significance (p)**
Total dietary fibre (g/100g)	-0.6849	moderate, negative	0.0289
Soluble dietary fibre (g/100g)	0.1910	no correlation	0.6625
Insoluble dietary fibre	-0.8167	strong, negative	0.025
OHC	-0.6800	moderate, negative	0.0305

WHC did not correlate with lycopene bioaccessibility from the model mixtures (r=0.0020; p=0.9966; data not shown), while higher OHC corresponded with a lower lycopene bioaccessibility. This is probably due to the more favorable formation of DF−lycopene complexes (Table 3). The data obtained also indicates that the length of the DF chain can also significantly influence formation of lycopene−DF complexes. The data of the LBC of pairs inulin - FOS and cellulose I - cellulose II clearly show that shorter chain length results in higher binding capacity. Namely, inulin is composed primarily of long-chain fructans with a degree of polymerization (DP) typically greater than 10 while FOS is a specific type of fructan that is shorter in chain length, with a DP ranging from 2 to 8 (data obtained from the producer). Similarly, in the case of cellulose, the average DF chain lengths of cellulose I and cellulose II are 75 μm and 180 μm, respectively, and this significantly influenced the binding of lycopene (Figure 2). The impact of particle size of the DF on lycopene bioaccessibility can be assessed by observing the differences in LBC of citrus fiber I (fiber size <30 μm) and citrus fiber II (fiber size 75 μm) that have the same chemical composition, but the citrus fiber II has slightly larger particle size. Our results show that the particle size of DF does not significantly influence lycopene bioacessibility since observed differences in lycopene bioacessibility from citrus I- and citrus II- mixtures were not statistically significant.

When trying to compare obtained data with the results of other studies, it becomes clear that these are very scarce and that most studies have been focused on fruits and vegetables that contain naturally present lycopene, leaving the exact insights into connections between DF structural characteristics and lycopene binding uninvestigated. Recent work of Feng and co-authors (2023) demonstrated the formation of complexes between lycopene and cellulose (as in our study) and showed that the cellulose network skeleton binds lycopene and improves its storage stability, thermal stability, and UV radiation stability. On the other hand, Gu and co-workers (2020) showed that in natural food matrices, SDF can bind significant amounts of lycopene. In their work, partial enzymatic degradation of SDF in tomato peel significantly improved extractability of lycopene. Similar observations were made by Li and co-workers (2022) who showed that the yield of lycopene from tomato pomace was raised by 57.2% after enzymatic treatment and partial degradation of DF.

In the formulation of foods for special medical purposes containing DF, the usual approach is to combine both SDF and IDF in adequate ratios. The DF composition is usually based on the recommendations for the intake of SDF and IDF for general nutrition or consistent with the guidelines for nutritional therapy of the particular health condition. Additionally, the impact of DF on textural and sensory characteristics of the product is also considered. In view of the above data, it would be useful to avoid the utilization of the DF with strong binding capacity for lycopene as it could negatively affect its bioaccessibility form the formulation.

However, it has not been investigated whether the data on LBC of the single DF can be used to approximate the LBC of the mixture of DF (with known DF composition). Namely, the synergistic or antagonistic effects of different DF within a mixture could lead to unexpected outcomes in the bioaccessibility of nutrients, indicating the need for comprehensive studies that consider the interactions within fiber mixtures rather than isolating individual components. Figure 3 shows lycopene bioaccessibility of experimental DF mixtures and compares obtained values with theoretically calculated based on LBC data obtained for single DF. Significant differences between obtained and expected (theoretically calculated) values indicate the possibility of synergistic or antagonistic interactions of DF.

Generally, bioaccessibility of lycopene from mixtures of DF was high, ranging from 71.8 ± 10.4 % in IN/FOS/DEX+CEL[1] mixture (containing the mixture of fructans and cellulose) up to 114.5±3.7 in MIX (containing the mixture of fructans and soluble DF). Comparison between experimentally determined lycopene bioaccessibility and the theoretical one shows that data on the interaction of lycopene with single DF can be used to predict lycopene bioaccessibility from the DF mixture. For example, the lowest lycopene bioaccessibility was assessed in the mixtures containing the highest amounts of cellulose (Table 1), and the effect of cellulose was higher in mixtures containing higher cellulose ratio (IN/FOS/DEX+CEL and IN/FOS/DEX+CEL[1]). However, it seems that the negative effects of IDF (such as cellulose) are often less pronounced in DF mixtures, as if the presence of SDF in the mixture decreases the possibility of IDF to bind lycopene. For example, the LBC of the DF mixture MIX (containing inulin, FOS, dextrin, gummi arabicum, citrus fiber and pea fiber) was lower than expected, indicating small but statistically significant solubility promoting effects of SDF. Also, the addition of cellulose to MIX decreased lycopene bioaccessibility to a lower extent, compared to theoretically determined level. Regardless of the observed differences, it can be concluded that data on the lycopene biding activity of the single DF can be useful in predicting the effects of DF mixture on lycopene bioaccessibility.

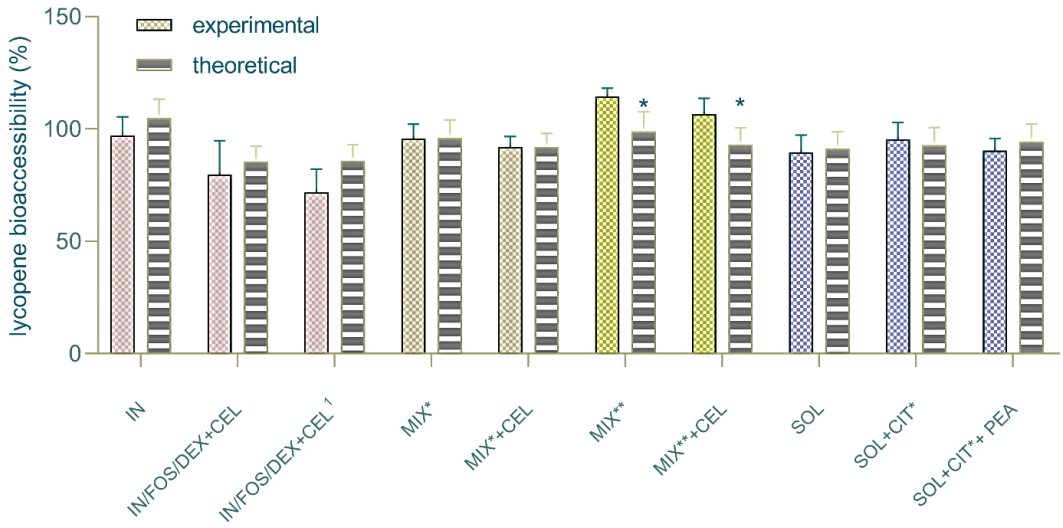

Fig. 3. Lycopene bioaccessibility from mixtures of dietary fiber (determined experimentally and calculated based on the composition of dietary fiber mixtures)
*Differences between experimental and theoretical lycopene bioaccessibility are statistically significant ($p<0.05$) indicating additive effects of dietary fiber in fiber mixture.

Conclusions

This study highlights the significant impact of DF and lycopene bioaccessibility, emphasizing the importance of understanding their interactions for the development of functional foods, particularly those intended for special medical purposes. The research demonstrates that different types of DFs bind lycopene to varying degrees, with SDF generally facilitating higher bioaccessibility compared to their insoluble counterparts. Obtained results show that the choice of DF in food formulations can be strategically optimized to enhance lycopene bioaccessibility, potentially offering opportunities for optimizing functional food formulations. However, additional research is required to confirm whether these effects lead to improved lycopene absorption and associated health benefits. Moreover, the study underscores the necessity for further investigation into the mechanisms governing DF−lycopene interactions. As the demand for functional foods continues to increase, a deeper understanding of these relationships will be essential to the development of effective dietary interventions to improve nutrient absorption and overall health outcomes.

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