

# **INFOGEST 2.0 digestion method: Characterization of the sterol content in the digestion reagents**

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The INFOGEST digestion method was adapted for the evaluation of sterol bioaccessibility in a plant sterol (PS)-enriched beverage [1]. Recent modifications of the method such as the addition of gastric lipase and cholesterol esterase, showed that the presence of sterols in the digestion reagents has an important role on sterol bioaccessibility [2]. However, the specific sterol contribution from these extracts is unknown. Therefore, in the present study, the sterol content of the extracts used in the INFOGEST 2.0 digestion method (rabbit gastric extract – RGE –, porcine pancreatin and bovine bile) is determined by GC-FID. Results show that cholesterol content of the extracts is as follows: bovine bile > porcine pancreatin > RGE ( $4.07 \pm 0.18$ ,  $1.41 \pm 0.07$  and  $0.57 \pm 0.05$  mg/g extract, respectively). Considering the amount of each extract added to the digestion, the greatest cholesterol contributor is porcine pancreatin followed by bovine bile and RGE ( $1.72 \pm 0.08$ ,  $1.00 \pm 0.04$ , and  $0.046 \pm 0.004$  mg, respectively). However, cholesterol in bile is found in pre-formed micelles that increase its solubility, and, therefore, it would compete for sterol micellarization against sterol provided by the digested

foods. This fact has been observed in previous studies in which the cholesterol content of the blanks of digestion is inversely correlated with PS bioaccessibility [2]. On the other hand, bovine bile contains stigmasterol,  $\beta$ -sitosterol and sitostanol ( $0.14\pm 0.01$ ,  $0.93\pm 0.07$  and  $0.18\pm 0.01$  mg/g extract, respectively), while porcine pancreatin contains campesterol, stigmasterol,  $\beta$ -sitosterol and sitostanol ( $0.103\pm 0.004$ ,  $0.25\pm 0.02$ ,  $2.17\pm 0.42$  and  $0.32\pm 0.01$  mg/g extract, respectively). Nevertheless, these PS present in the extracts appear in negligible amounts in the digestion blank, reflecting their low solubility *vs.* cholesterol. In conclusion, in order to optimize the INFOGEST 2.0 gastrointestinal method for the evaluation of sterol bioaccessibility, the characterization of the sterol content in the digestion reagents provides valuable information since it may condition their solubility.

**References:** [1]. Blanco-Morales et al. *Food Funct*, 2018, 9, 2080-2089; [2]. López-García et al. 2021, Book of abstracts “Virtual International Conference on Food Digestion”, pp 44 (<https://www.cost-infogest.eu/content/download/4051/35805/file/V-ICFD%20Book%20of%20Abstracts.pdf>)

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