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Complementarity of clinical trials, model systems, and metabolomic workflow to unravel the healthy effects of foods: BEBESANO *vs* MODELSANO: A case study

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Abstract

Nowadays, the health benefits associated with the consumption of plant-based food constitute a hot topic. To further demonstrate such benefits, related to antioxidant, anti-microbial, and anti-inflammatory activities, as well as the reduction of the risk of several pathophysiological conditions, the study of bioaccessibility and bioavailability of specific food's constituents, which require interdisciplinary networks, is essential. In this frame, although different experimental models can be developed, the workflow described in the present work support the application of intervention trials in humans as the first option to study the truly effects on health of foods (e.g., plant-based foods), due to the safe condition of them and the realistic approach of this kind of studies, later explored in depth resorting to *in vitro*, *ex vivo*, and pre-clinical models, as the most appropriate workflow to get reliable results in the field of Food Science and Nutrition, regarding mechanisms of actions and molecular interactions. Thereby, the work described in the present review is developed in the frame of two consecutive and interconnected projects: BEBESANO (concluded) and MODELSANO (in process) that demonstrate the efficiency of the workflow proposed for research in the Food Science and Nutrition fields. In this regard, in the frame of BEBESANO, acute and longitudinal interventions in humans, devoted to set-up bioavailability of bioactive compounds, followed by functional studies *in vivo* upon pre-clinical models were conducted to unravel the relationship between bioactive compounds in plant-based beverages and the use of sweetener replacer. Now, most relevant findings from BEBESANO are being further explored in the newly granted project MODELSANO, which is aimed to uncover gaps of knowledge about the mechanisms behind the descriptive results obtained in BEBESANO, using more restrictive *in vitro* models (allowing the development of studies on the cellular and molecular pathways involved), and integrative cutting edge mathematical modelling alternatives.

Keywords: *In vivo*; *in vitro*; bioavailability; bioaccessibility; bioactivity; health-promoting foods; metabolomic; mechanistic studies.

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Introduction

In recent years, global awareness on the health benefits associated with the consumption of plant-based food (fruits, vegetables, and manufactured products) have increased. These benefits have been stressed as a result of broad research describing the biological activity of bioactive nutrients (peptides, fatty acids, vitamins, minerals, and so on) and non–nutrients (phytochemical compounds) present in edible and by-products of vegetable origin, namely radical scavenging capacity, antimicrobial, and anti-inflammatory activities, and contribution to lowering the risk of an array of pathophysiological non-communicable conditions, including cancer, obesity, type II diabetes mellitus, and cardiovascular and neurodegenerative diseases, among others [1]. Among the bioactive compounds pointed out as responsible for such biological benefits, major attention has been paid to (poly)phenols, which have been related to diverse health claim, mainly associated with cardiovascular health [2].

Nonetheless, despite the cumulative evidence of the biological benefits associated with the dietary intake of plant-based foods, to take advantage of the biological properties of such bioactive compounds, further insights on their bioaccessibility and bioavailability, especially regarding the concentration that could be achieved in target cells and tissues, remains essential. To enhance the current knowledge about the health benefits of plant-based foods and their constituents, unravelling factors involved in the bioaccessibility and bioavailability of the compounds of interest, which are critical factors for retrieving valuable biological effects, along with their effect on health within the frame of certain pathophysiological processes, needs to be further explored. With this objective, setting up interdisciplinary networks is critical to address questions affecting complex biological systems. Thereby, experimental approaches to unravel the relationship between food intake and its effects on health, besides interdisciplinary contribution, needs of diverse perspectives and experimental models to get complete and consistent results [3]. This would allow taking advantage of the blessings of the different experimental alternatives and thus, shed light, not just on the benefits for the health of specific dietary interventions, but also on the molecular mechanisms and interactions affecting the bioavailability of bioactive compounds, as well as the interactions of such molecules with key elements of the cellular metabolism and molecular pathways, finally responsible for the healthy properties.

So, given the safety inherent to foods, the workflow that should be applied to characterize their benefits for health is suitable to start with dietary interventions in humans, which provide valuable information on the actual biological functions of the bioavailable fractions of bioactive compounds and their impact on biomarkers of a range of pathophysiological conditions, that would allow identifying dietary habits capable to enhance the health status. Afterwards, basic research resorting to model systems *in vitro* and metabolomic approaches would provide additional evidence to fully understand the mechanisms of action and, as a result, the feasibility of applying joint interventions/treatments (even a combination of dietary and pharmacological approaches) to obtain the benefits expected reducing the administrations of drugs.

In some detail, human intervention studies allow establishing the effect of foods and/or their bioactive components on diverse pathophysiological conditions, as well as to explore the bioavailability of the distinct food's components of interest and thus, to identify those molecules absorbed intact or metabolized towards bioactive derivatives [4]. However, these models are not the final solution for all research needed to get a complete characterization of a given dietary intervention, since present several constraints, mainly associated with the gap of knowledge associated with these approaches on the molecular mechanisms responsible for intestinal absorption, spread over the diverse tissues and cell types, and the biological activity monitored. Even more relevant, clinical trials are enclosed to much relevant factors that difficult the interpretation of the results and the decision making process, such as the inter-individual variation, environmental factors, lifestyle, complex organization, ethical considerations, and poor compliance by subjects or drop out of subjects [5]. Because of all these limitation factors, human research should be complemented by the development of additional models that cover the weakness referred to.

In this way, once established the benefits of specific foods, to get further insights into the mechanisms of action, an array of *in vitro* models has been developed, for instance, concerning bioaccessibility and bioavailability, to study the impact of the gastrointestinal settings on the bioavailability of bioactive compounds present in plant-based foods. According to gastrointestinal physiology, these models have been divided theoretically into digestion and colonic fermentation models [6], each of them providing complementary information on the effect of the diverse stages on the stability of phytochemicals. The former (regarding both static and dynamic modes) mirrors, *in vitro*, the functioning of the gastric and small intestine chemical/enzymatic digestions, providing very useful information on the capacity of the digestive process to release food constituents, as well as on structural changes of interest under the physicochemical conditions during digestion [7]. On the other hand, models of *in vitro* colonic fermentation have been set to study the relative involvement of the microbiota metabolism in the bioavailability of bioactive compounds native in the plant material or their metabolized forms into additional bioactive derivatives and thus, to get further impact on the health of plant-food constituents [8]. However, as mentioned concerning human interventions, *in vitro* approaches are not exempted of limitations. The major constraint associated with *in vitro* models is the weak of representation of host functionality, although it was suppressed, to some extent, by the development of *artificial digestive systems* that can simulate the different human digestive functions [9].

Concerning bioavailability, *in vitro* models are represented by a range of cell cultures techniques that constitute an *in vitro* tool to study the intestinal absorption of bioactive compounds, by using human or animal tissues to further understand the ratio of absorption of the compounds of interest, under controlled environmental conditions, being complementary to the bioavailability models *in vitro*. Bioavailability *in vitro* models are also associated with severe drawbacks, mainly associated with the poor representation of the tissues structure, cells proliferation, differentiation, and interconnection between cell/tis-

sue types, as well as their rapid degradation [10]. To overcome these limitations, *ex vivo* techniques have been developed. These involve a whole/part of living tissues and lead to more controlled experimental conditions [11].

Because of the constraints associated with the diverse experimental models described (clinical trials, *in vitro* models, and *ex vivo* techniques), an array of pre-clinical (animal models) approaches has been developed, representing a valuable alternative that allows filling the gap between *in vitro* studies and clinical/dietary interventions in humans [5]. The main advantages of turning to pre-clinical models are the capability to set studies at almost any stage of their life cycle, the minimization of the variability dependent on genetic traits and environmental factors due to the use of "inbred" experimental animals, maintained under strongly controlled housing and health conditions, so-called "Defined Animals" [9].

Once exposed to the main experimental models, the question arises about which is the most appropriate sequence of experimental models to establish and go on through the characterization of the effect of a given food/treatment on health. *In vivo* models are the ones providing outcomes closest to reality, and despite the constant evolution of *in vitro* models and the improvement of their capacity to simulate the physiological events, *in vivo* models still cannot be replaced as easy. This fact, together with the safe condition of foods, turn intervention trials in humans as the first option to shed light on the effects on health of foods that allows identifying gaps of knowledge on mechanisms of action and molecular interactions that should be further explored resorting to *in vitro*, *ex vivo*, and pre-clinical systems. Indeed, this is the workflow followed in the frame of the projects BEBESANO and MODELSANO that is used in the present article as an example of the advantages that could be retrieved from the different models, in the field of Food Science and Nutrition.

BEBESANO: A project devoted to identifying the benefits of newly developed plant-based beverages upon clinical trials

This project (BEBESANO-*New beverage, rich in bioactive compounds, to modulate the Energy metabolism in overweigh adults*) was envisaged as a result of the awareness of the results provided by the currently available epidemiological studies that have evidenced the existing relationship between the consumption of sugary drinks and the risk of diverse non-communicable diseases, namely obesity, type II diabetes mellitus, cardiovascular disease, and the metabolic syndrome [12,13]. This type of beverage, generally made with sucrose and fructose syrup as sweeteners, are sources of rapidly absorbable sugars that entail a rapid increase in plasma glucose and insulin after ingestion, thus constituting risk factors for the development of metabolic diseases and initiating insulin resistance and intolerance to glucose. Consequently, recently, searching for new non-caloric sweeteners that could be used as an alternative to the conventional ones, helping to reduce the sugar intake without compromising the organoleptic features of drinks has emerged as a hot topic that focused on the aims of the project. Research developed to date has been suggested that this would help to reduce important risk factors regarding obesity and diabetes, as well as several cardiovascular and neurologic diseases [14].

Simultaneously to the sugar-related health issues, and its association with the beverages industry, in recent years, an augmented consumption of fruits and vegetables in the population, more and more aware of the effect of this kind of foods exert on health, has been experienced.

Based on these antecedents, the combination of both the necessity to lower the sugar intake and to raise the consumption of plant-based foods (or beverages) in the frame of balanced diets, has allowed facing the challenge of developing new formulations for healthy plant-based foods that preserve the content of bioactive nutrients and non-nutrients, while reducing the energy content of vegetable drinks. This is the first objective of the research project BEBESANO, for which a dietary intervention in humans was identified as the most appropriate experimental approach to gain knowledge on the relationship between the sweetener and the pharmacokinetics, bioavailability, and biological activity of bioactive molecules present in the plant-based beverages newly designed and developed. In this frame, it was hypothesized that the achievement of this objective would allow providing alternatives to decrease the excessive sugar intake and counteract the postprandial response linked to sugar consumption in overweight people.

To contrast the starting hypothesis, two paradigmatic fruits, lemon (*Citrus limon*) and maqui-berry (*Aristotelia chilensis*), dietary sources of bioactive (poly)phenols (mainly flavanones and anthocyanins, respectively), were chosen for the design and development of a new functional drink featured by a pleasant aroma and high nutritional/phytochemical [15]. Anthocyanins have been shown to improve hyperglycemia and insulin sensitivity *via* activation of diverse cell signalling pathways [16] and, the combination of maqui berry and lemon, allows obtaining very stable beverages in terms of color and (poly)phenolic content [17]. Once obtained the dietary source of phenolic the taste was fine-tuned using sucrose or alternative non-caloric sweeteners (sucralose and stevia) to explore the beneficial effects of consuming a sugar-free, (poly)phenols high, drink, depending on the sweetener used, on the pharmacokinetics and bioavailability of the bioactive compounds of interest (anthocyanins and flavanones) and the effect on postprandial hyperglycemia.

The appellation to nutritional intervention trials by BEBESANO to contrast the starting hypothesis was addressed by recruiting a well-characterized population and the use of the metabolomic techniques that resulted in a very valuable election. In this regard, due to the complexity of associating the biological benefits attributed to a given food with the bioactive compounds responsible for such actions, through the leak of information about their absorption and metabolism, this type of characterizations requires the identification of reliable biomarkers, measured in complex biological samples [9]. At first, these markers included ingestion biomarkers in plasma and urine samples collected by minimally invasive sampling procedures, and give rise to the identification of circulating metabolites resulting from the ingestion of (poly)phenols, as well as biomarkers of inflammatory processes, oxidative stress, lipid, and glucose metabolism. The analysis of all these markers provided evidence of the beneficial effect of the dietary intervention under characterization regarding clinical parameters associated with type II diabetes mellitus and the identification of the bioactive molecules (native of the maqui-citrus beverage or derivatives) responsible for such activities.

The most relevant information retrieved from this project could be classified into three main topics: bioavailability upon acute interventions, longitudinal interventions, and biological activities, which are further described in the following sub-sections.

Bioavailability upon acute interventions

Acute bioavailability studies, also known as dose-response or postprandial studies, are frequently performed, as a preliminary approach, to enhance the knowledge about the factors affecting the pharmacokinetics and bioavailability of bioactive compounds. This model provides very valuable information on the biomarkers (native molecules in the food matrix and its phase II and microbiota metabolism derivatives) that turn it into an essential approach for the further characterization of the effect of chronic ingestions upon longitudinal studies in terms of bioavailability and biological activities. Complementarily, the application of acute administration strategies can be also implemented in the frame of pre-clinical assays, resorting to which information on the quantitative profile of the bioactive compounds in the separate tissues and cell types, can be obtained.

Regarding acute intervention studies, in the frame of BEBESANO project, this model allowed monitoring the concentration of anthocyanins and flavanones of maqui berry and citrus beverages (and their metabolic derivatives) achieved in peripheral blood plasma as preliminary information on the shelf-life of these compounds when ingested by diet, and the influence of the diverse sweeteners employed on intestinal absorption. Also, excretion in 24-hour urine of these compounds provided detailed information on their bioavailability. So, this approach provided, resorting to pharmacokinetics determinations, evidence on a valuable intestinal absorption rate for these compounds, exception made of anthocyanin and hesperidin aglycones, as their pass through the digestion system courses with the formation of a variety of phase II derivatives. The study of the pharmacokinetics of the different (poly)phenols present in the maqui berry citrus beverages evidenced that the highest intestinal absorption of most compounds and the metabolic derivatives (caffeic acid, 3,4-dihydroxyphenylacetic acid, naringenin, *trans*-ferulic acid, and vanillic acid) was achieved when ingesting drinks developed using sucralose as sweetener, followed by stevia-based beverages [18-20].

Concerning assessments on urine samples, most results retrieved pointed out sucralose and stevia as valuable replacers of sucrose that lead to the greatest bioavailability of bioactive anthocyanins and flavanones and their derivatives (caffeic acid, catechol, 3,4-dihydroxyphenylacetic acid, eriodyctiol, homoeriodyctiol, hippuric acid, naringenin, trans-ferulic acid, 2,4,6-trihydroxybenzaldehyde, trans-isoferulic acid, and vanillic acid) for most metabolites, thus suggesting the beverages under consideration as an interesting dietary source of molecules previously demonstrated on biological activities and preventive potential against the above referred to pathophysiological conditions [18,19]. The metabolites identified were phase II derivatives and reached the highest urine concentration at 3.5 hours after the intake of the newly designed maqui-citrus beverages [18,19]. So, this first intervention demonstrated that the amount of the diverse bioavailable metabolites excreted in urine when ingesting beverages developed using stevia and sucralose as sweeteners surpassed the values provided by sucrose that could be caused by a tentative interaction of the diverse sweeteners on the intestinal absorption mechanisms for flavanones and anthocyanins [18,19]. In this sense, and according to the descriptions available in the literature on the mechanisms responsible for the intestinal absorption of (poly)phenols, the different bioavailability provided by the alternative sweeteners could be attributable to the central role of intestinal sugar transporters in the absorption of flavonoids, as attached sugar in esterified phenolics condition strongly their solubility in the intestinal mucus. Besides, the competence events that could be established between the separate sweeteners used and the phenolic compounds found could be related to these differences in bioavailability [20]. However, at this moment, this explanation is, to some extent, speculative, and need for additional research resorting to complementary models (*in vitro* or *ex vivo*) to further demonstrate the truthfulness of the statement.

Longitudinal interventions

Longitudinal or chronic studies should be also established to obtain a complete picture of the impact of a given dietary intervention on human pathophysiology. This type of study completes the information already recorded from the acute one since allows evaluating ulterior modifications of the bioavailability parameters (e.g., as a result of the tissue distribution), thus informing on the stable circulating concentrations due to the long-term administration of the dietary source of the bioactive compounds of interest. Also, longitudinal studies inform on the actual modification of bioactivity markers, such as parameters related to oxidative stress or inflammation, as well as long-term hormonal response. Again, the use of reliable biomarkers of bioavailability and bioactivity is essential to extract robust conclusions from longitudinal interventions.

According to these premises, in the frame of the BEBESANO project, to move forward from the results obtained from the acute intervention study, as well as to explore possible accumulative effects of the (poly) phenols present in the maqui-citrus beverages, a longitudinal intervention was developed [21]. This research also monitored the effect of the long-term ingestion (2 months) of the developed beverages on peripheral-blood plasma and urine concentrations of the target bioactive (poly) phenols. This study reinforced the results and conclusions retrieved from the acute intervention, leading to identify a diversity of metabolites, including phase II derivatives, synthesized as a result of the metabolism of intestine epithelium cells, hepatocytes, and microbiota on the original bioactive compounds present in the new maqui-citrus products. Again, the absence of parental anthocyanin and hesperidin aglycones after the long-term intake of the beverage was observed, further suggesting the rapid metabolism of these molecules by phase II reactions. Moreover, it was observed that the metabolites identified were persistent over time [21]. Urine samples assessed exhibited the following molecules: caffeic acid, catechol, 3,4-dihydroxyphenylacetic acid, eriodictyol, homoeriodictyol, hippuric acid, naringenin, *trans*-ferulic acid, 2,4,6-trihydroxybenzaldehyde, *trans*-isoferulic acid, and vanillic acid derivates [21]. Regarding plasma samples, caffeic acid, 3,4-dihydroxyphenylacetic acid, eriodictyol, homoeriodictyol, hippuric acid, naringenin, 2,4,6-trihydroxybenzaldehyde, and vanillic acid derivates were detected. Besides, the results obtained reinforced the findings described resorting to the acute interventions described in the previous section, with the identification of stevia as the sweetener providing the highest bioavailability for most metabolites. Moreover, these results evidenced that after 2-months of ingestion of the beverage, the amount of the different metabolites increases, and more metabolites are detected in comparison with the acute intake of the maqui-citrus beverage that would indicate, to some extent, accumulative effects might occur, even though the bioavailability of these metabolites was similar regardless the sweetened-drink ingested [21].

Sorting out biological activities *in vivo*

As referred to regarding bioavailability studies, when researching in the field of food science and technology and nutrition, "Preliminary" *in vivo* research to identify valuable bioactivities and preventive capacity against diverse pathological conditions, in humans, is more important than establishing mechanisms of action on speculative bioactivities, which are not confirmed at all, as a result of dietary ingestions. Indeed, this should be considered the first step to affirm or deny the theoretical health benefits, tentatively attributable to the intake of specific foods, as a result of their content bioactive compounds, even though additional mechanistic studies would be still required. Moreover, although previous studies have described specific effects, *in vivo* model studies are needed to fill the gap of knowledge about the effect focusing research.

Thereby, once established the bioavailability of the bioactive compounds present in maqui-citrus drinks resorting to both human acute and longitudinal studies, the analgesic properties of maqui berry, the main ingredient of the new drinks, were assessed, to contrast the hypothesis suggested, based on the use of these plants for healing, nervous system disorders, pain, and inflammation, among others [22,23]. So, accordingly, the next challenge tackled in the frame of BEBESANO was to study, through *in vivo* (pre-clinical) models, neurological properties of the bioactive compounds present in the maqui berry citrus drink, as well as in the separate ingredients used during its design and development (maqui berry extract, citrus extract, and their combination, with and without the addition of sweeteners (sucrose, sucralose, and stevia). In this regard, the capacity of maqui berry administrated by orogastric (gavage) and parenteral (intraperitoneal) routes to alleviate pain by attenuating the nociceptive sensitivity was studied upon a mouse (Swiss Webster) model [24]. This procedure allowed retrieving evidence of a dose-dependent antinociceptive activity, independently of the route of administration. The antinociceptive activity observed was due to the development of efficient biological activities during both neurological and inflammatory phases, thus suggesting actions at the central and peripheral levels of the nervous system [24], tentatively due to the achievement of high concentrations of maqui berry's phenolic compounds (mainly represented by anthocyanins) in nervous tissues. However, as referred to regarding the bioaccessibility and bioavailability evidence obtained in the course of the project, these outcomes need further mechanistic studies by applying, most likely, a balanced combination of *in vitro* and pre-clinical approaches [24].

MODELSANO: A further insight into the mechanisms responsible for the bioavailability and bioactivity of bioactive compounds characterized in BEBESANO

As described in the previous section, the accomplishment of the objectives planned in BEBESANO, besides the relevant information retrieved on the bioaccessibility, bioavailability, and effect on health, some pertinent questions raised regarding the molecular interactions between the components of the beverages developed, with the diverse sweeteners employed, and with specific receptors in different cell types in humans. These interactions would be responsible for the different bioavailability and anti-nociceptive activities described, and cannot be clarified using only in vivo trials. To answer these questions, it is essential to go to additional and complementary models, represented in this case by model systems and in vitro approaches devoted to cellular and molecular biology, using a metabolomic workflow as the common thread that would help to interconnect the complex data network generated.

So, to advance towards a further insight into these interrogations, the Project MODELSANO (*Modelling the processing and health benefits of citrus-maqui beverages. influence of sweeteners*) was envisaged (currently in course). This new project strip from the referred to questions raised by BEBESANO and, accordingly, is aimed at unravelling the effect on the stability of bioactive compounds of the maqui berry-citrus fruits of industrial processing, as well as on clarifying the chemical reactions triggered by the addition of sweeteners, which may, in turn, influence the drink's metabolome and thus, the bioaccessibility, bioavailability, and bioactivity of the (poly) phenols present in the beverages characterized, and determine what factors must be considered to achieve the ultimate goal of obtaining an effective healthy drink on postprandial hyperglycemia, to provide actual alternatives to excessive sugar intake and counteract the postprandial response linked to sugar consumption in subjects with low levels of chronic inflammation, that complements the results obtained for the biological activities on volunteers. However, these questions cannot be tackled by intervention studies *in vivo*, since would require invasive procedures that are ethically unacceptable. Therefore, it is necessary to apply model processing systems and develop mathematical tools to describe them.

Concerning the gaps of information on the factors affecting the different bioavailability obtained depending on the sweetener applied, the research team in charge of this new project understand that the application of a more restrictive model to study bioaccessibility and bioavailability (*in vitro* model) would be of interest to get complementary information. In this regard, the application of *in vitro* techniques would allow obtaining biological samples corresponding to specific stages and thus, drawing the molecular events giving rise to the effect of the gastrointestinal digestion conditions on the bioaccessibility, stability, bioavailability, and bioactivity of (poly)phenols by applying target and untargeted metabolomic approaches.

On the other hand, once identified the anti-nociceptive effect associated with the oral administration of the maqui-berry citrus beverages, the application of *in vitro* models allows establishing an experimental design resulting of the partial or complete digestion processes and bioavailable fractions of the digestates. This would provide a collection of data available to be further analysed resorting to an array of mathematical tools developed to identify correlations between composition and function (target metabolomics) and to identify additional derivatives of the bioactive compounds present in the maqui-berry citrus drinks, as well as new markers of pathophysiological conditions that could be affected by the exposition to such bioactive phytochemicals (untargeted metabolomics). These studies propose a useful tool to find new metabolites associated with the use of different sweeteners added to the beverage, providing valuable information on the metabolic pathways affected.

At this point, it is very relevant to stress that mathematical modelling and data analysis are central elements to enhance the scope of both descriptive and mechanistic research, contributing to demonstrate relationships between *a priori* independent events and to optimize processes. This requires multidisciplinary research to attain valuable results, that will allow obtaining predictions, simulate scenarios without depending on experimental work, estimate uncertainties, or design and optimize processes. As a consequence, MODELSANO (*in vitro* modelling systems) is focused on solving the issues arisen from

BEBESANO (development of new foods and dietary interventions in humans).

Conclusion

The present review deals with the selection of the most suitable experimental models and workflow to enhance the knowledge about the relationship of specific foods and/or their constituents and health. Accordingly, intervention trials in humans are proposed as the first option due to the safety of foods and the proximity of them to the reality, followed by *in vitro*, *ex vivo* and pre-clinical systems to fill emerging gaps of the first trials.

BEBESANO and MODELSANO are consecutive projects that exemplify the proposed workflow that allows addressing the objectives shared between them, which should be divided theoretically because of operative reasons. So, upon the first project, acute and longitudinal interventions in human were set, followed by *in vivo* mouse experimental models, to study the bioavailability and bioactivity of the bioactive compounds of a new developed maqui-citrus beverage, and provided promising results about the bioavailability of anthocyanins and flavanones and the neurological action of the former ones. Afterwards, the results retrieved from the first project allowed drafting a complementary proposal (also granted), MODELSANO, which emerges as a continuation project to cover the gaps of knowledge identified in BEBESANO thus providing information on the mechanistic facts responsible for the main results on bioavailability and bioactivity attained previously, by the use of more restrictive *in vitro* models, mathematical modelling, and data analysis alternatives.

In this way, once the choice of the sequence of experimental models to be used has been justified, we can conclude that setting up intervention studies in humans, *in vivo* and *in vitro* models, in that order, could be the properly experimental design to tackle very relevant challenges in the field of Food Science and Nutrition.

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