Protocol for the development and validation of an online Fermented Foods Frequency Questionnaire (3FQ) for the assessment of fermented foods consumption patterns across European regions

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Submitted to: JMIR Research Protocols
on: November 24, 2024

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Abstract

Background: Fermented foods vary significantly by food substrate and regional consumption patterns. While consumed worldwide, their consumption and potential health benefits remain understudied. Europe, in particular, lacks specific consumption recommendations for most fermented foods.

Objective: This project, which is under the framework of the PIMENTO COST Action (CA20128), aims to develop a validated tool to quantitatively estimate fermented food intake across European Regions.

Methods: Fermented foods were categorized into broad groups (e.g., dairy, plant-based, meat, beverages) based on product classifications, ensuring that the foods included were genuinely fermented through ingredient analysis. Participants aged 18+ were recruited online via snowball sampling. They were asked to provide informed consent and agree to data collection under ethical guidelines using a GDPR-compliant platform.

Results: A representative sample of 1536 participants per European region was targeted, ensuring diversity in age and sex, with the goal of achieving a 60% response rate. A multilingual questionnaire was developed and pilot-tested for clarity. The upcoming steps will include final validation for accuracy and repeatability using 24-hour dietary recalls and specific statistical techniques of analysis to ensure reliability.

Conclusions: The validated online-based Fermented Foods Frequency Questionnaire (3FQ) aims to address current gaps in fermented food intakes to help improve future research in this important area.

(JMIR Preprints 24/11/2024:69212)
DOI: https://doi.org/10.2196/preprints.69212

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Protocol for the development and validation of an online Fermented Foods Frequency Questionnaire (3FQ) for the assessment of fermented foods consumption patterns across European regions

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Abstract

Background: Fermented foods vary significantly by food substrate and regional consumption patterns. While consumed worldwide, their intaken and potential health benefits remain understudied. Europe, in particular, lacks specific consumption recommendations for most fermented foods. **Objectives:** This project, which is under the framework of the PIMENTO COST Action (CA20128), aims to develop a validated tool to quantitatively estimate fermented food intake across four European Regions. **Methods:** A specific Food Frequency Questionnaire for fermented foods was designed to quantify their intake in terms of frequency and quantity. Fermented foods were categorized into broad groups (e.g., dairy, plant-based, meat, beverages) based on product classifications, ensuring that the foods included were genuinely fermented through ingredient analysis, using the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus for fermented foods as a guide. For each main fermented foods group, subcategories were included following elaborate discussions of the scientific expert-panel and country-specific examples were given; for example, for hard cheese, Parmigiano was chosen in the Italian version, and Graviera in the Greek. The questionnaire was developed in English (universal version) and was then translated in multiple languages using back-translation method. Each version was pilot tested for clarity and data for the prospective validation were gathered. This included two key steps: assessing repeatability by having participants retake the questionnaire after six weeks, and confirming accuracy by comparing

3FQ results against 24-hour dietary recalls from a subsample of participants. Statistical analyses will be used to confirm agreement between the methods. Representative sample calculations were performed for 4 groups; by biological sex and age group (18-49.9 and 50+). To ensure representative sample obtainment, participants aged 18+ were recruited online using multiple social media platforms, in all countries, snowball sampling, and potential supplementation with panels through the survey platform. Prior to all responses participants were asked to provide informed consent and agree to data collection under ethical guidelines using a GDPR-compliant platform. **Results:** A representative sample of 1536 participants per European region was targeted, ensuring diversity in age and sex, with the goal of achieving a 60% response rate. A multilingual questionnaire was developed and pilot-tested for clarity. The upcoming steps will include final validation for accuracy and repeatability using 24-hour dietary recalls and specific statistical techniques of analysis to ensure reliability. **Conclusions:** The validated online-based Fermented Foods Frequency Questionnaire (3FQ) aims to address current gaps in fermented food intakes to help improve future research in this important area.

Keywords: fermented foods; consumption data; intake; human health; European regions.

Introduction

The fermentation process involves the action of microorganisms indigenously present in the food (raw) material or added as starter culture(s) under adequate environmental conditions [1]. In the past years, the potential health benefits of fermented foods, microorganisms contributing to the fermentation process, and the resulting fermentation metabolites, vitamins and bioactive compounds have raised great interest. [2, 3] with the potential for improving human health [4, 5] in children and adults [6]. Nevertheless, assessing the potential health benefits of fermented foods remains a challenge since over 5000 fermented foods and beverages are produced globally [7], and many remain understudied. Moreover, there are no specific recommendations for their consumption in Europe [8] since specific consumption data are limited and addressed mainly in overall dietary assessments.

One must consider the variety of substrates, products, and microorganisms involved to understand the diversity of fermented foods and beverages [9]. For instance, the same substrate, milk, can be the basis for most of the available fermented dairy products (e.g., yoghurt, kefir, and cheese) [10], and although the same substrate is used, the final products differ considerably in terms of nutritional composition and bioactive compounds. Also, the fermentation process may involve the action of one or more microorganisms that are either indigenously present in the raw material, added as starter cultures or via back-slopping and grown under appropriate environmental conditions that may or may not be alive at the time of consumption [1, 11]. Moreover, the microorganisms used for fermentation are associated with different metabolic pathways and reactions involved in the process, leading to various physicochemical transformations in the final products. For example, different groups of microbes may produce different end-products (metabolites), including organic acids (e.g., lactic, acetic acid) via various metabolic pathways under different conditions [12]. Also, while some microorganisms are known for their capacity to synthesize specific vitamins (folate, riboflavin, and cobalamin) from various precursors in plant and dairy foods [13], others do not influence or even consume vitamins [14]. Additionally, various fermented foods have been reported to contain a series of bioactive peptides deriving through the action of proteases synthesized by fermenting microorganisms [8]. Consecutively, it is not surprising that fermentation from a nutritional perspective is also associated with the reduction of antinutritional factors and the improvement in the overall nutritional value and digestibility of fermented foods [15].

Despite their potential health benefits, fermented foods are generally not recommended as a category in dietary guidelines, and in fact, there are no specific recommendations for their consumption in Europe [8, 13]. A challenging factor is the fact that the type of fermented foods consumed varies by region and by countries in specific regions [8]. Differentiation by main food substrate is necessary to understand and assess the potential health benefits of fermented foods and acquire ample information to make specific recommendations. As discussed above, these food substrates may vary significantly regarding nutritional composition and products formed during fermentation; therefore, population consumption data are necessary to understand their role in human health.

To date, there remain limited quantification methods, developed for assessing specifically fermented foods intake in populations. A recent study identified and estimated fermented foods consumed in Japan using dietary recalls by season and found 1396 unique fermented foods consumed [16]. In the Dutch observational cohort (NQplus) that used a food frequency questionnaire (FFQ), an estimated 17% of foods consumed were fermented, and another 13% were included in composite dishes [17]. The validation results showed that FFQs could effectively estimate foods and beverages that are regularly consumed, such as coffee, bread and cheese, since the mean difference between recalls and the FFQ was small. However, this was not the case for fermented foods consumed more sporadically [17]. Both studies support the hypothesis of the variety of fermented foods available, regardless of the food substrate, with the importance of developing a tool that will assess intake of the food substrates and a vast majority of their main byproducts being augmented, as a first main step to understand their action on human health. In this aspect, another recent study evaluated 'food intake biomarkers' for various fermented foods to discriminate the dietary patterns of fermented foods [18]. Although this is of great value, many biomarkers can be affected by human metabolic rates (concentration biomarkers) and, therefore, do not accurately reflect absolute food intake. Therefore, estimating frequency and quantifying fermented food intake using validated dietary assessment methods are also necessary [19], as well as developing tools that minimize their potential limitations [20]. One of the main limitations is an accurate quantification, which can be addressed using validated colour food atlas pictures, where each icon refers to specific portion intake (weights), and the individual selects the usual portion from a series of pictures instead of one [15] or of subjectively expressing an estimate of quantities consumed. This process [21], along with using food models or selected house measures when no valid food picture is available, has been found to increase intake accuracy [22]. Nevertheless, because fermented foods represent a specific type of food intake, a tool that explicitly evaluates the consumption of these foods and depicts specific examples by substrate and their potential subcategories can help address the limited information reported for these foods. Reckoning intake in a representative population may help validate types of biomarkers for further research on fermented foods and health.

The main aim of the questionnaire is to collect and analyze fermented foods consumption, using predefined food groups and their substrates, in the four main European Regions, as defined by EuroVoc [23]. The project is carried out as part of the PIMENTO COST Action (CA20128) [24], with the aim of creating a universally validated tool for recording the intake of fermented foods of European consumers.

Methods

The research protocol of this study was approved by the Ethics Committee of the Agricultural University of Athens (27/05.05.2023).

Identification and classification of fermented foods

Identification and classification of fermented foods can be challenging as there are multiple definitions and perceptions of what constitutes and what is not a fermented food. Towards this, in 2019, an expert panel by the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined fermented foods as "foods made through desired microbial growth and enzymatic conversions of foods" [23]. This definition encompasses a wide range of products that may or may not contain live microorganisms, and it was used for this questionnaire.

Considering this, the breadth of fermented foods that exist in the marketplace and the diet of different European consumers, fermented foods were first stratified in the Fermented Foods Frequency Questionnaire (3FQ) into broad food groups, namely plant-based products, dairy products, legumes, meat and/or fish, vegetables, cereal products, chocolate, non-alcoholic beverages, vinegar, coffee, tea, chocolate beverages, beer and/or cider, wine and spirits. These food groups were defined *a priori* and were based on the classification of product categories taken into consideration for mapping fermented foods within the activities of WG2 of the PIMENTO CA20128. Fermented foods within each food group were then aggregated into subgroups. A series of exclusion criteria were also applied to ensure that the studied foods were indeed fermented. Foods that were traditionally fermented but are often no longer fermented because of contemporary food processing (such as pickled vegetables) or foods that were partially fermented (such as green or black teas that are typically oxidized rather than post-fermented) were included or eliminated based on the ingredient lists of commonly consumed grocery store products. Moreover, and with the exceptions of chocolate and some plant-based alternatives, partially fermented foods (i.e. salad dressings) and composite dishes (i.e. chocolate-based confectionery) were not considered. For example, sauerkraut is made from cabbage, water and salt and its sour taste and preservation are achieved through lactic acid fermentation by live bacteria, which aligns with the study's definition of a fermented food, whereas pickled gherkins made from cucumbers, water, vinegar, salt and sugar get their sourness and acidity directly by the added vinegar, not by microbial activity. Therefore, this product was excluded as it is pickled but not fermented.

Study sample

The study aimed to have a representative sample for each of the four European Regions. A predefined sampling frame was formulated, and the STEP procedure was followed to calculate the minimum sample size required for each European Region for an 80% study power and with a 95% confidence level. A response rate of 60%, as reported by many epidemiological studies, was accounted for, and a potential 10% attrition rate. The goal was to achieve a representative sample for four groups: males and females, young adults (18-50 years), and older adults (≥ 50 years of age). Based on a 5% margin of error and a conservative approach of 50% for the indicators to be assessed, a total sample of 1536 from each European region was required (for four groups). This translated to a target of 2560 contacts, based on the 60% anticipated response rate. The representativeness by group has been monitored (quota monitoring) throughout the study process based on European Region population distribution response rates. In situations where representativity for certain quotas (region, age group, sex) was not achieved, the online platform selected provided the option to use predefined panels from specified areas and required characteristics. Specifically, the platform could be used to target sampling from predefined panels in underrepresented areas and specific age groups. The predefined panels could also be used to rectify imbalances in the sex distribution, as evaluated during the development of the sampling frame and representativity, for each European region.

Responses obtained through this method have been integrated with the originally collected data, ensuring a cohesive and representative final dataset that aligns with the aims of our study; however, sensitivity analyses will be conducted (with and without the extra data from the panels) to evaluate potential response differences that also impact results. Initially a comprehensive comparison of the essential demographic, socioeconomic, and lifestyle attributes of participants recruited online against those from predetermined panels will be performed to assess if there are substantial variations in parameters that could affect the primary outcomes documented consumption patterns of fermented foods—between the two groups. A primary analysis using the complete, integrated dataset will be performed followed by a secondary analysis using exclusively the non-panel sample. The two results will be compared to ascertain whether the incorporation of panel data significantly modifies the study's conclusions. The outcomes of these sensitivity studies will be fully disclosed in the publication.

Inclusion/exclusion criteria

Participants were considered eligible for participation in the study when they were above 18 years of age at the time of recruitment and agreed to the informed consent form. The survey was conducted online, and the questionnaire was hosted on the online platform Conjointly. Subjects were recruited through the use of many channels: (a) the PIMENTO website (https://fermentedfoods.eu/); (b) social media (i.e. LinkedIn, Facebook, X, Instagram, etc.); (c) emailing past survey participants for whom we have contact information; (d) through information from notified sites and non-profit organizations; (e) and through snowball sampling where respondents were encouraged to forward the invitation to any interested parties.

Conjointly is an online research platform used in this study to recruit participants and administer the 3FQ. It is General Data Protection Regulation (GDPR) accredited with an option not to maintain IP addresses increasing the study’s confidentiality. It was chosen since it provides researchers with integrated tools to build and customize surveys using a wide range of question formats, from simple scales to complex experimental designs. It also generates QR codes automatically and is accessible through smart phones (IOS and Android). The platform codes responses automatically as uploaded, and provides the total database coded and with summary statistics for rapid data evaluation during the recruitment phase and sample data collection, reducing selection bias. It also supports advanced analytical methodologies, which is used to statistically measure consumer preferences.

Each participant was asked to provide informed consent before proceeding to the survey. The informed consent included a detailed explanation of the study's main aim, including how data will be collected, stored and used and the potential risks that accommodate online questionnaires. It also underlined that the participant is not obliged to respond to all questions and that they can leave the study with no consequences at any time. For the validation process (second part of the study), where personally identifiable information was asked to be provided, participants were asked to re-consent again in order to be contacted. Additionally, participants selected from predefined panels, participated in the primary survey only, and were ineligible for the validation phase of the 3FQ, thus segregating the validation outcomes from this possible source of bias. The study protocol has been pre-registered in the Open Science Framework, a registry that accepts observational studies as recommended to help decrease publication bias [25]. The process abides by the ethical principles for research involving humans, as reported by the Declaration of Helsinki [26].

Questionnaire

Respondents were initially asked to answer general questions, including self-reported anthropometric data. Also, the respondents answered undemanding questions regarding their health state and any allergies, as these parameters may affect the knowledge and behaviour of fermented food consumption. Participants were then presented with the 12 fermented food categories and were asked to choose whether they consume each of these food categories and, through visualizations, indicate their usual consumption portion. The included fermented food categories were plant-based meat alternatives, dairy products, legumes, meat, fish, vegetables, cereal products, chocolate (bars), non-alcoholic beverages and alcoholic beverages (beer and/or cider, wine, and spirits), vinegar, and beverages (coffee, tea, chocolate beverages). Although this classification is rather extensive, as discussed earlier, some food groups included in the 3FQ structure may not be widespread nor frequently consumed in all countries, which is the main challenge as well as the necessity of this multi-European region study in order to identify differences in the amount and type of fermented foods consumed in each region.

Questionnaire development and validation

The Questionnaire was firstly developed in English by experts in food science and technology, nutrition, nutritional epidemiology and consumer science. National Contact Points (NCPs; up to two) from each country were responsible for translating the questionnaire into their language, formulating it identically to the originally derived international tool (English version). Each country, however, used nationally available food examples per main food group question to personalize the questionnaire (for example, in the cheese group, Greece used feta, and Italy used mozzarella as soft/semi-soft cheese examples). Countries with more than one national language translated the questionnaire in all (for example, Switzerland translated in French, Italian and German used in their country, other to the national translations from the respective countries). A standardized translation methodology was followed, which included two steps: (i) the translation to the national language by an individual fluent in the national and English languages and (ii) the back-translation from the national language to English, with the individual responsible for the second step being blinded to the primary international questionnaire. The back-translated questionnaire was compared to the international questionnaire, and necessary corrections were made.

The clarity of the questionnaire was checked through pilot testing. This was performed through the Conjointly platform, as per methodology, using 50 individuals of various age groups and educational backgrounds. The participating individuals were asked to keep notes of difficulties they faced accessing or responding to the questions and/or potential clarifications required. These were reviewed by the NCP members, and corrections were included in the final version.

The 3FQ will also be validated, a process that includes two parts. The first part is to assure the repeatability of the 3FQ across all European regions, and the second to assess accuracy in total. For repeatability, participants were asked if they would be willing to repeat the same questionnaire approximately 6 weeks after their first response. Those participants who responded positively were asked to consent and to provide an email address and or phone number, for further contact at a later stage. A minimum of 200 responders from each European region were calculated as necessary to be targeted within each region to assess repeatability, following the 5 responders per question rule of thumb.

The second part of the validation will assess the accuracy of the 3FQ using a harmonized methodology. This process will use participants from the total sample of the participating countries. To maximize the harmonized process NCP's accepting to act as interviewers were

trained by a dietitian specializing in nutritional research methods through two online one-hour sessions and one-onsite workshop. A specific excel file was also developed using drop down menus and was shared to all interviewers to complete during the recall process of each individual.

For the actual procedure, participants were asked if they consented to share their communication details (as previously described) and be contacted for an online interview of their usual dietary habits. The individuals who shared either a phone number or email address were contacted no later than two weeks after completing the online 3FQ to undergo a 24-hour phone-based recall. The validation process includes one or two 24-hour phone-based recalls, using the Automated Multipass Method, from a representative sample of the study target population [27]. A total of 265 to 371 individuals is required for this process, as per rule of thumb (5-7 participants per survey question). Study power calculation was performed for the Spearman correlation analysis using the G*Power application. Specifically, calculation of the sample required to achieve 90% power, assuming a low correlation of ρ_o=0.4 (the Null Hypothesis H_o) with the potential of no correlation (the althernative Hypotheis H_1=0.2), at alpha=5%, two-tailed exact test, showed that 218 participants were required. Due to the fact that only 1 recall would be most probably obtained from most participants, and that Bland-Altman analysis requires more data, a larger sample based on the 7 participants per question rule was targeted, in order to random error. The larger number (371) was further aimed for, as most optimal, since this ensures adequate power to assess the accuracy of the subproducts of each food group as well. Participants who did not provide dietary assessment data or complete at least one recall will be excluded from the validation dataset. At least one recall was required (and up to two were performed) for the validation of the 3FQ's accuracy. This criteria was chosen since the aim was not to assess overall usual intake but to validate the 3FQ through a meaningful measure of fermented food intake. Permitting collection of recalls on one or two days can help reduce participant burden, hence decreasing selection bias of working individuals. Also, it was clearly noted that the recall would be interview-based to help reassure and decrease selection bias of older participants, although this population is overall more hesitant with online processes. Measures were taken to ensure a representative sample for the 3FQ responses, specifically including an older age group representative sample (of 50+) for the 3FQ responses. For the questionnaire's validation, demographics will be disclosed to depict the population that results can be justifiably generalized to." After merging the 3FQ and 24-h recall data, the population sample will represent the validation subsample for further analyses.

Data on fermented foods from the 3FQ and recalls will be identified and aggregated into conventional food groups. Percent differences in mean intakes, quintile cross-classification, Spearman's correlations, and Bland-Altman analyses will be used to evaluate the agreement between the two dietary assessment methods.

Results

The project is part of the PIMENTO COST Action (CA20128) but does not receive specific funding, other to member travelling and accommodation costs during on site working groups. The questionnaire has been developed, translated, and pilot-tested, with the main data collection phase started in October 2023 and completed in May 2024 The validation phase, which will include repeatability testing and 24-hour dietary recalls, is scheduled to be completed in December 2024. Data analysis is set to start in January 2025 with the results anticipated for publication 4 months post analysis commencement.

Discussion

The study project handles a variety of operational, methodological, and moral ethical concerns, particularly in relation to the use of online surveys. A significant limitation is the exclusion of susceptible demographics like children and adolescents and individuals without legal capacity, reducing the generalization of the results. Furthermore, there are advantages and disadvantages to performing online surveys. An important advantage that is an asset pertaining to the study's aim is the ability to reach a sizable, geographically diversified sample at a reasonable cost. It also allows the flexibility to allow respondents to finish the survey at their leisure. The GDPR-certified platform enables automated data collecting and processing that will help minimize human error misclassification. Online surveys have certain drawbacks, such as selection bias, that must be considered. For example, people without internet access or low technological proficiency may not be included, resulting in an under-representation of demographics. Furthermore, response rates might be lower than in-person techniques due to difficulties in understanding the question. To deal with this obstacle, different types of pilot testing were conducted before the study launch: 1. The survey was launched to a panel of food science researchers and nutritionists to complete the survey and comment on areas that require correction and/or explanation. Feedback received was reviewed by the study's co-principal investigatos (PI), then discussed with the NCPs, and improvements/adjustments were made. 2. Difficulties encountered during the survey's pilot phase by each NCP using a convenience panel from the general population were also discussed, and further adjustments were made to improve clarity. Privacy issues pertaining to the management of anthropometric, health, and personal data are also of great importance, especially when creating profiles. Data will be anonymized and securely stored, with restricted access to authorized personnel. Only the absolute necessary anonymized personal data will be stored for a maximum of five (5) years at the Agricultural University of Athens, after which the PIs will carefully dispose of it.

In the situation where predefined panels were used because representativity and certain quotas were not met, sensitivity analyses will be conducted, and all significant differences between the two groups (based on the collection method) found from comparisons and tests performed will be highlighted. If the results are consistent, this will strengthen the study and its validity. If the results differ, the data will not be combined, the results will be separately presented, and a detailed discussion on the implications will be derived as necessary. A weighted approach to balance the influence of each data source may be used if differences are minimized. The results will be ethically handled and disseminated to safeguard participant confidentiality while effectively informing interested parties, including the public, academics, and policymakers. Lastly, despite the challenges recognized, careful planning and ethical supervision are entailed to ensure that the study significantly contributes to the field of nutrition and food science, for the necessary next actions to take place.

Limitations:

While we cannot completely eliminate the potential for social desirability bias, especially for foods perceived as “healthy” the methodology used aimed to mitigate reporting errors. Specifically, the Automated Multipass Method for the 24-hour recalls was used to minimize misreporting and improve recall accuracy through systematic probing, and the 3FQ was designed with a hierarchical structure that included broad food groups followed by specific fermented subgroups. This was used as a “filter” to systematically guide participants' repsonses and help reduce perception errors. Furthermore fermented foods like kefir, kombucha, or

kimchi are distinctive and are often consumed with intention or due to cultural reasons, which can lead to more accurate reporting compared to more commonly consumed items. The potential of under-representing individuals of lower literacy level or those with no-computer or internet access should also be considered. The platform selected was accessible through a smart phone as well either android or ios, easily accessed through a QR code, potentially mitigating selection bias of individuals with no computer access. As per the effect of literacy level, it is plausible that the subject matter itself, consumption of fermented foods, may have an inherent selection effect towards a more literate population hence potentially reducing the bias introduced by an online survey tool compared to a survey on a more general topic. Future analyses could address this by statistically weighting the data against national census statistics using demographics on literacy level of the population. This information has been included in the Methods section (where the Conjointly platform is described) and the limitations section (for the literacy level and prospective possibilities).

Conclusion

The online 3FQ is designed to address existing research gaps in assessing frequency and quantity of fermented foods consumed, considering all main food groups. This will enable researchers to gain deeper insights into dietary patterns involving fermented foods and support more robust analyses of their potential associations with health outcomes, while also enhancing the accuracy and comparability of data in future studies. This specific tool will also be able to address consumption data of less frequently consumed fermented foods that are regularly missed in usual food frequency questionnaires, hence minimizing systematic errors of consumption.

Author Contributions: MS, GV conceptualization of study; EM study methodology; EM, SK, TS, MS, formulation and writing of protocol; SK, SA, KJB, VKS, ML, VM, TS, GV, BV CC, reviewing of protocol.

Acknowledgements: The authors sincerely thank all National Contact Points (NCPs) who helped disseminate the questionnaire (in alphabetical order): K. Adamberg (EST), D. Ağagündüz (TUR), M. Beglaryan (ARM), D. Borch Ibsen (DNK), E. Brouwer Brolsma (NLD), J. Burtscher (AUT), M. Cerjak (HRV), K. Chandolias (SWE), Z. Ciesarová (SVK), I. Ciproviča (LVA), F. De Filippis (ITA), E. Delia (ALB), M. Deschasaux (FRA), A. Gamero (ESP), M. Gandia (ESP), A. Giertlova (SVK), M.C. Grech Perry (MLT), L. Hoxha (ALB), D. Jaros (DEU), H. Jenssen (NOR), P. Jones (MLT), A. Kalea (GBR), B. Kalyoncu (TUR), J. Karl (IRL), L. Kennes (NLD), G. Kostov (BGR), A. Kusar (SVN), M. Laranjo (PRT), E. Mantzari (GRC), N. Mikulec (HRV), E. Mudura (ROU), N.E. Nagybakay (HUN), L. Ove Dragsted (DNK), L. Ozola (LV), N.A. Pancevska (MKD), P. Papademas (CYP), D. Pipoyan (ARM), I. Pravst (SVN), H. Rohm (DEU), P. Russo (ITA), T. Sar (SWE), M. Starowicz (POL), I. Talkic (BIH), B. Trajkovska (NMK), A. Vukojevic (SRB), M. Wronkowska (POL), G. Nakov (BGR), T. Pohjanheimo (FIN).

Funding: The authors received financial reimbursement from the PIMENTO COST action (CA20128) for the meetings and workshops conducted during tool preparation. MS received a grant to launch the questionnaire to specific populations.

Conflicts of Interest: None declared. SK is alone responsible for the content and views expressed in this and they do not represent the decisions, policy, or views of the Hellenic Food Authority.

Abbreviations:
3FQ: Fermented Foods Frequency Questionnaire
FFQ: Food Frequency Questionnaire
NCPs: National Contact Points
ISAPP: International Scientific Association for Probiotics and Prebiotics

Data availability: The study protocol has been uploaded to the Open Science Framework (https://osf.io/ud4jf/). Data collected will also be included and open after an embargo period of 3 years. Database(s) used for a publication will be made available, without details on individual patient data, post manuscript acceptance.

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Supplementary Files

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