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## D4.2

### REPORT ON USE CASES AND TNA PROVISION

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|----------------------------|--|
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## Table of contents

|   |           |
|---|-----------|
| <b>1. Executive Summary .....</b>   | <b>3</b>  |
| <b>2. Introduction .....</b>  | <b>4</b>  |
| <b>3. General description of the use cases.....</b>                         | <b>5</b>  |
| <b>4. General timeline of the use cases.....</b>                            | <b>8</b>  |
| <b>5. Evaluation of applicants for transnational access.....</b>            | <b>9</b>  |
| <b>6. Description, planning and follow-up of the use case projects.....</b> | <b>17</b> |
| <b>7. Discussion .....</b>  | <b>27</b> |
| <b>8. User engagement and satisfaction.....</b>                             | <b>32</b> |
| 8.1 User engagement.....  | 32        |
| 8.2 User satisfaction .....   | 32        |
| 8.3 Socio-economic impact.....  | 32        |
| <b>9. Conclusions and plans for the next steps .....</b>                    | <b>34</b> |
| <b>List of abbreviations .....</b>  | <b>35</b> |
| <b>References .....</b>   | <b>36</b> |
| <b>Annex 1 - User Satisfaction Survey.....</b>                              | <b>37</b> |

## 1. Executive Summary

METROFOOD-RI - Infrastructure for Promoting Metrology in Food and Nutrition ([www.metrofood.eu](http://www.metrofood.eu)) is a distributed Research Infrastructure (RI) aimed to promote scientific excellence in the field of food quality and safety. It provides high-quality metrology services in food and nutrition, comprising an important cross-section of highly interdisciplinary and interconnected fields throughout the food value chain, including agri-food, sustainable development, food safety, quality, traceability and authenticity, environmental safety, and human health. It combines a Physical-RI (P-RI) and an electronic-RI (e-RI) for open data deposition, access, and processing. The P-RI coordinates and integrates an existing network of state-of-the-art facilities including: in the “Metro” side, laboratories for the full chemical, physical-chemical and microbiological characterisation of foods and any matrix of interest in relation to the agri-food (e.g., environmental matrices from the agroecosystem of production, feeds, food contact materials, etc.), and plants for Reference Material (RM) development and production; in the “Food” side, experimental fields/farms for crop production and animal breeding, small-scale plants for food processing and storage, kitchen-labs for food preparation, and “demo” sites for direct stakeholder engagement (e.g., to run Living Labs). The e-RI consists of a service-oriented electronic architecture providing an accessible platform for sharing and integrating data, knowledge, and information on metrological tools for food analysis and for facilitating the availability and use of agri-food data to the user community. The e-RI collects, integrates, and makes the P-RI results open and interoperable, organising and complementing them with existing data and providing tools for various uses of the data, even promoting their interoperability and the integration with data arising from other existing networks and infrastructures. METROFOOD-RI users are individuals, teams or institutions who are foreseen to use the services of the RI. Four main user categories have been identified as follows: Researchers and academic communities; Policy makers/food inspection and control agencies; Food business operators (FBOs); Consumers/citizens. METROFOOD-RI is structured according to a *Hub & Nodes* model. The Central Hub (CH) will be the statutory seat of the ERIC and will represent the heart of the strategy, coordination, communication, and administration of METROFOOD-RI, coordinating the overall infrastructure and managing the central e-portal, which will give access to all the resources and services of the infrastructure. The CH will act as a coordinating European layer across all National Nodes (NNs), while the NNs will represent the operational sites of the infrastructure.

METROFOOD-EPI (GA 101130162) is the Horizon Europe funded project supporting the Early Phase Implementation of METROFOOD-RI. Its overarching mission is to advance the building process of METROFOOD-RI as infrastructure consolidated for its full implementation and ensure an effective start of the operational phase.

This deliverable focuses specifically on the process and provision of Transnational Access (TNA) services, a core component of METROFOOD-RI's mission to foster collaboration and promote metrology in food and nutrition research. By integrating services from a variety of institutions, METROFOOD-RI aims to ensure that researchers, policymakers, food business operators, and consumers alike can access cutting-edge analytical tools and metrological services across a highly interdisciplinary spectrum. This report highlights the steps taken to facilitate access to these services, providing a thorough evaluation of how institutions across different countries collaborated to offer integrated solutions.



## 2. Introduction

TNA plays a crucial role within METROFOOD-RI, enabling researchers and industries to access – among others - advanced analytical services across different facilities and countries. Two use cases have been selected to exemplify the complexity and collaborative nature of the project, providing a real-world test of how well the integrated services from various institutions across different countries can function in practice:

- **Use case 1:** Service 1 - Characterization of fermentation processes and their bio-based derived products from the food industries
- **Use case 2:** Service 2 - Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products

It is particularly important for assessing the effectiveness, feasibility, and coordination of these services across a broad network of institutions.

Building on lessons learned from the METROFOOD Preparatory Phase (METROFOOD-PP, GA 871083), METROFOOD-EPI introduces a higher level of service integration and complexity. While METROFOOD-PP identified the foundational challenges of coordinating within institutions, METROFOOD-EPI seeks to refine those processes by further integrating services and testing more complex, cross-disciplinary applications between institutions. Involving multiple institutions for each use case presents both opportunities and challenges, allowing for a comprehensive evaluation of how well these integrated services work in real-world applications, while requiring careful coordination to overcome logistical, technical, and communication barriers. By integrating services from multiple countries and institutes, METROFOOD-RI challenges the existing infrastructure to work cohesively, ensuring that transnational collaborations are effective and that services can be delivered smoothly across borders and disciplines.

This deliverable focuses on the entire TNA process, outlining the steps involved in planning, managing, and following up on the use cases. It highlights the timeline for each case, the coordination efforts between service providers, and the continuous monitoring required to ensure smooth progress. Special attention is given to the challenges encountered, the strategies used to overcome them, and the successes that demonstrate the value of the project. A user satisfaction survey will also be sent to participants to help assess the impact and efficiency of the services and identify areas for improvement. Furthermore, this phase aims to explore how scalable these integrated services can become, assessing their potential for future implementation on a broader scale across the infrastructure. The outcomes of these use cases will also impact a wide range of stakeholders, from researchers to food businesses and policymakers, helping to enhance food safety, innovation, and sustainability across Europe. Overall, this report aims to demonstrate how well the METROFOOD-RI performed under real-world conditions, focusing on the collaborative and logistical aspects of TNA provision, and offering recommendations for further enhancing the integration of services across countries and institutions.



### 3. General description of the use cases

Two specific use cases have been selected for this phase of METROFOOD-RI to test the integration and efficiency of TNA services. A detailed description of the services related to each use case is provided below.

#### **Use Case 1: Characterisation of fermentation processes and their bio-based derived products from the food industries**

The first use case focuses on the characterisation and safety testing of the fermentation processes and bio-based products derived from them. The service is designed for use in food processing and packaging and aims to provide high quality scientific expertise and technical solutions to help industrial food business operators and researchers improve and adapt their food fermentation methods and final food-derived products.

Fermentation has been a cornerstone of food production for centuries, contributing to the development of a wide range of food products, from bread and beer to yogurt and soy sauce. However, as the demand for sustainable, safe, and nutritious food grows, there is a pressing need to refine and optimize these processes to meet modern standards (1, 2). To address this, the service offers a consolidated approach to studying fermentation, encompassing microorganism identification through metagenomics to identify key microorganisms responsible for product quality, safety, and bioactivity. Using advanced tools such as metabolomics, metatranscriptomics, and metaproteomics, the service can further characterise these microorganisms at the molecular level. This enables researchers and industry stakeholders to precisely control fermentation variables like temperature, pH, and nutrient levels, optimizing the safety, quality, and health benefits of the final product. Moreover, fermentation can lead to the production of bioactive compounds with health-promoting properties, making it a valuable tool in the development of functional foods.

With increasing concerns over foodborne illnesses and the safety of food packaging materials, ensuring both the microbial and chemical safety of fermented products is essential. This use case can further address these concerns by providing comprehensive services that assess not only the microbial profile but also the chemical and omics composition of raw material and end-product. Additional services include contaminant detection, biopolymer characterisation, migration studies, toxicity analysis, and bioactivity testing of end products.

Overall, this use case provides an extensive range of services provided by EUT, IJZSRM, INSA and TUBITAK, that addresses the complexity of the food fermentation processes. It offers in-depth analysis for a broad array of stakeholders, including academic researchers and companies involved in food fermentation. By integrating cutting-edge methods, the service ensures that food fermentation processes can be optimised in line with industry demands, ultimately improving product safety, functionality, and quality.

A visual representation of the service provision is available in Figure 1.

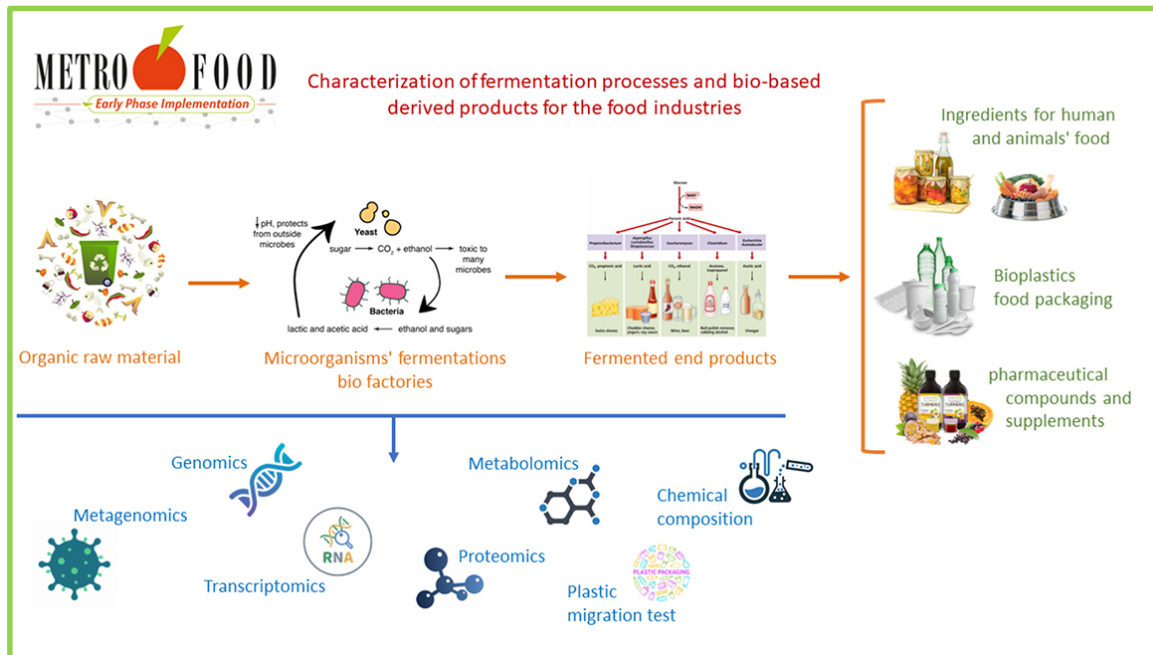


Figure 1 – Graphical abstract of use case 1.

### Use Case 2: Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products

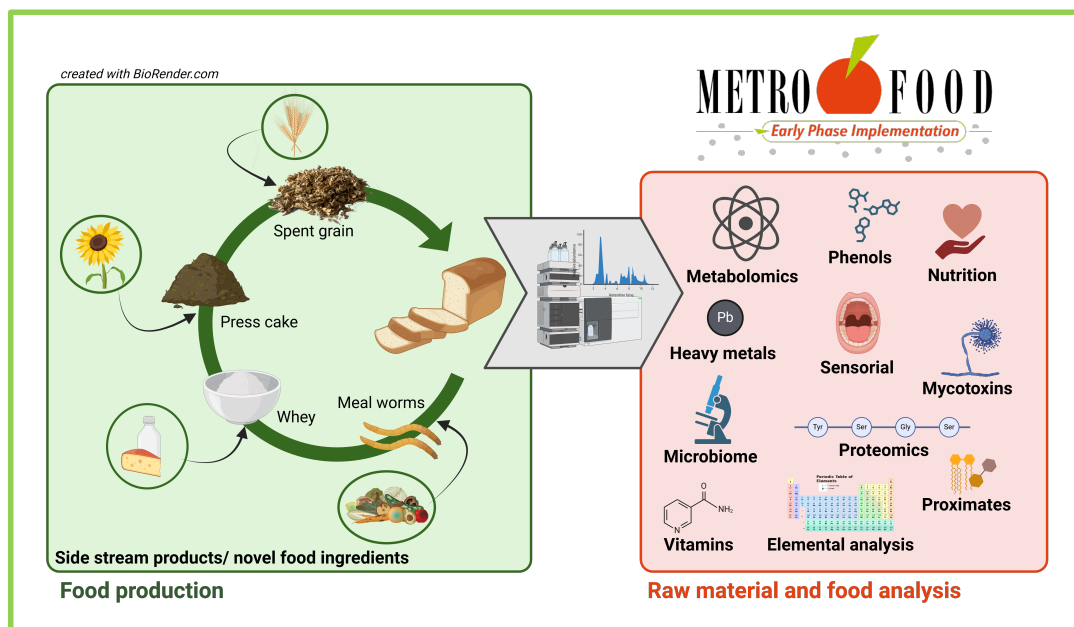
The second use case aims to integrate advanced food technologies and analytical services for the development of new food components and products using by-products from food processing, such as cereals, and novel ingredients, including insects. This use case is particularly focused on promoting sustainability in the food industry, contributing to circular systems that reduce food waste and increase resource efficiency.

Insects, as an alternative protein source, present a highly sustainable and nutrient-dense food option, rich in protein, vitamins, and minerals. Compared to conventional livestock, insect farming requires less resources and produces fewer greenhouse gas emissions. Embracing insect-based foods can diversify food sources, reduce environmental impact, and enhance food security for present and future generations (3). Additionally, the use of side-stream products from food processing, offers an opportunity to further improve resource efficiency (4). These side-stream products are found throughout the agrifood sector, such as whey from cheese production, sunflower press cakes from oil extraction, and spent grains from brewing, each still containing promising amounts of nutrients. Many different stakeholders, from agriculture to food manufacturing, could profit from the valorisation of these by-products by including them into processes such as baking (5). For the successful development of these novel and sustainable food ingredients, the technological design should be strongly intertwined with a comprehensive chemical-physical characterisation, assessing sensory attributes and nutritional value, to enable product safety, usability, as well as transparency for producers and consumers.

As such, the project aligns with the growing movement toward a circular economy, a system aimed at reducing waste and pollution by keeping materials in use for as long as possible. Circular

economy practices have gained significant momentum in recent years, both in scientific research and political discourse. With more than 13,000 publications referencing this concept, it has become a key aspect of the European Union's future strategy (6). By reducing food waste and creating value from by-products, this project contributes to a more sustainable, resource-efficient food system that can meet the challenges posed by food insecurity, environmental degradation, and climate change. To address these challenges, this use case features an integrated service approach provided by several key institutions: TUM, IBA, AUTH, ENEA, CZU, and JSI leveraging their expertise and resources.

A visual representation of the service provision is available in Figure 2.



**Figure 2** – Graphical abstract of use case 2.



#### 4. General timeline of the use cases

The services were launched through open transnational calls on a dedicated section of the METROFOOD-RI website ([www.metrofood.eu/access/open-call.html](http://www.metrofood.eu/access/open-call.html)), providing opportunities for external users to access the infrastructure. ENEA, as the coordinating institute, SCIENSANO, leading WP4 – *Access and service offer consolidation*, and PREMOTEC, leading WP3 – *e-component and data management solutions*, were responsible for announcing the calls, setting up the application form, and managing the user registration in the internal database. The open call content, scientific background and the project concept were prepared by the lead service providers EUT and TUM. The open calls were promoted through various channels, including – among others – the METROFOOD-RI website and social media platforms and the websites of the individual partner institutes.

To ensure a transparent and secure process, the registration system and data collection procedures were designed in compliance with the General Data Protection Regulation (EU) 2016/679. Clear application and eligibility conditions were outlined for prospective applicants. While the service provision was offered free of charge, costs related to travel, lodging, and subsistence were not covered for the applicants.

Potential users were invited to apply through an online application form created using LimeSurvey. Each application was carefully reviewed by the respective service providers based on predefined criteria. The evaluations were then submitted to the Management Committee of METROFOOD-EPI for final approval within two weeks after the closure of the open calls. Successful applicants were notified within a month. Throughout the service provision, users and service providers maintained active communication, ensuring smooth execution of the planned experiments. At the end of the service provision, a survey was distributed to users to gather feedback on their experience, contributing to the continuous improvement of the service offering. Table 1 presents a general timeline for the two use cases, outlining the key phases from the launch of the open calls to the conclusion of the service provision.

**Table 1** – General timeline of METROFOOD-EPI use cases

| Month   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| <b>Characterisation of fermentation processes and their bio-based derived products from the food industries</b>   |   |   |   |   |   |   |   |   |   |    |    |    |
| Open Call   |   |   |   |   |   |   |   |   |   |    |    |    |
| Evaluation & notification   |   |   |   |   |   |   |   |   |   |    |    |    |
| Execution   |   |   |   |   |   |   |   |   |   |    |    |    |
| Results reporting   |   |   |   |   |   |   |   |   |   |    |    |    |
| <b>Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products</b> |   |   |   |   |   |   |   |   |   |    |    |    |
| Open Call   |   |   |   |   |   |   |   |   |   |    |    |    |
| Evaluation & notification   |   |   |   |   |   |   |   |   |   |    |    |    |
| Execution   |   |   |   |   |   |   |   |   |   |    |    |    |
| Results reporting   |   |   |   |   |   |   |   |   |   |    |    |    |



## 5. Evaluation of applicants for transnational access

The open calls for the two use cases (dedicated to TNA access) were open to all external users allowing them to register and access an application for each of the two services separately. The call remained open until April 20<sup>th</sup>, 2024 (M4). After the closure of the open calls, METROFOOD-RI received 8 applications for the *“Characterisation of fermentation processes and their bio-based derived products from the food industries”* and 5 applications for the *“Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products”*. The applications were forwarded to the respective service coordinators, EUT and TUM, for further evaluation and selection of the final candidates.

For both use cases, the evaluation process began with a preliminary screening, where incomplete applications were eliminated from further consideration. Only those applications that met the basic submission requirements proceeded to a more detailed two-phase evaluation process. Phase I consisted of a preliminary pre-screening based on four “knockout” criteria, while Phase II involved a more detailed evaluation using nine criteria, with a rating system from 1 to 5 points.

### **Phase I: Pre-screening**

The following criteria were applied during the pre-screening phase to assess the suitability of the applications:

1. **Compliance with the schedule**, to ensure that the proposed activities align with the project timeline is crucial for maintaining the overall coordination and meeting the METROFOOD-EPI deadlines. Applications that do not adhere to the schedule risk disrupting the flow of the project and are therefore screened out.
2. **Relevance to the open call and overall METROFOOD-RI objectives**, to ensure that each project aligns with the initiative’s mission of fostering innovation and sustainability in food safety and quality, thereby maximising the impact of the research.
3. **Technological and logistical feasibility**, to assess whether the proposed projects could realistically be implemented given the available resources, infrastructure, and technologies. Projects that lacked practical feasibility would face significant execution risks, making them unsuitable for further consideration.
4. **Scientific and technological excellence**, to ensure high-quality contributions and demonstrate a solid foundation in scientific research or technological advancement, which is essential for driving the development of sustainable food systems.

### **Phase II: In-depth evaluation**

After the Phase I pre-screening process, the remaining applications went through a more comprehensive evaluation in Phase II. The following criteria were applied during the in-depth evaluation phase to assess the suitability of the applications:

1. **Previous experience** in related fields, to ensure the applicant has the necessary expertise to successfully execute the project. This criterion helps to assess the applicants’ capacity to deliver high-quality results based on a proven track record.
2. **Selected service**, to ensure that the proposed services are available and feasible within the scope of METROFOOD-RI, confirming that the necessary resources and capabilities were in place to support successful project execution.



3. **Project relevance** to both the specific objectives of the open call and the broader METROFOOD-RI mission to ensure that the proposed work would have a meaningful contribution.
4. **Project objectives**, to assess the direction and potential impact of the project and make sure that the proposed objectives are achievable, aligned with METROFOOD-RI's goals, and designed to deliver measurable outcomes.
5. **Project timing**, to verify that the work can be completed within the designated timeframe, and fits within the broader METROFOOD-EPI schedule and milestones.
6. **Samples**, to assess the quality and quantity of the samples to be used in the project to ensure that the project design is scientifically robust and that the samples are appropriate to generate meaningful results.
7. **Scientific relevance**, to determine its potential contribution to advancing knowledge in the field and to ensure that the project has a solid foundation in rigorous scientific methodologies, with the capacity to provide meaningful and valuable insights.
8. **Technological relevance and feasibility**, to verify that the project could be practically executed with the existing technological infrastructure and that it can drive technological innovation within the scope of METROFOOD-RI's objectives.
9. **Sustainability and economic benefit**, to assess that the project would not only provide scientific and technological advancements but also promote sustainable practices and generate economic value in the food sector.

A summary of the description and evaluation process by the respective service providers is presented in the next sections.

### Characterisation of fermentation processes and their bio-based derived products from the food industries

The evaluation and rating process was carried out by the institute overseeing use case 1 (EUT), in coordination with the other partner institutes. A total of eight applications were submitted and eligible for the pre-screening. Below are the reasons for project application exclusion or inclusion from Phase II, based on the Phase I criteria.

#### **Application 1: Metabolomic and metagenomic analyses of marine algae tempeh and oncom, a traditional fermented food of Indonesia.** (University, Indonesia)

- While officially meeting timeline requirements, the project faced feasibility challenges due to logistical constraints, complexities in distributing samples over long distances, and differences in national observances, affecting coordination.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.

→ Application 1 was rejected



**Application 2: From waste to value. Volatile fatty acids production of brewer's spent yeast for its valorisation into PHA.** (Food business operator - Industry, Spain)

- The proposed timeframe was not compatible with the METROFOOD-RI timeline, making the project unfeasible.
- Lack of relevance to the open call and misalignment with the broader objectives of METROFOOD-RI. While this project focused a valuable process for waste valorisation, it was not directly related to food fermentation or the improvement of food-derived products.
- Technological and logistical challenges, particularly due to the extended timeframe required, made the project impractical.
- Insufficient scientific and technological foundation, with no supporting publications provided.

→ Application 2 was rejected

**Application 3: From waste to value valorisation of brewer's spent yeast: PHA bacterial pure production from volatile fatty acids obtained through acidogenic fermentation.** (Food business operator - Industry, Spain)

- The proposed timeline did not align with the METROFOOD-RI schedule, making it ineligible for evaluation in Phase II.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Insufficient scientific and technological foundation, with no supporting publications provided.

→ Application 3 was rejected

**Application 4: Characterise and evaluate the potential as food ingredient or as a raw material for extracting phytochemical compounds.** (Food business operator - Industry, Portugal)

- The project was not compliant with the schedule, as the requested analytics were extensive and not aligned with the call's specific requirements.
- There was strong relevance to the open call and overall METROFOOD-RI objectives.
- Technological and logistical aspects were deemed unfeasible.
- Demonstrated solid scientific and technological excellence.

→ Application 4 was rejected

**Application 5: Feasibility and safety evaluation of bio-based plastics for beverage industries versus traditional PET packaging.** (Soc. Coop. Agr., Italy)

- The project was not compliant with the schedule due to a lack of detailed information.
- Strong relevance to the open call and aligned well with METROFOOD-RI objectives.
- Insufficient information from the supplier to properly assess the project's feasibility. The project lacked clarity on sample details, including number, type, and specific analytical procedures required.
- High level of scientific and technological excellence.

→ Application 5 was rejected



**Application 6: Proteomic and metabolomic characterization of the fermentation product *Bacillus subtilis* natto DG101 (SME, Spain)**

- The proposed timeframe was feasible.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.

→ Application 6 was included in the phase II in-depth evaluation

**Application 7: Unravelling the microbial diversity and lipidome profile of Spanish-style and natural cv. Chalkidiki green table olives throughout fermentation and 12-month storage under low NaCl conditions at industrial scale: application of multi-omics approaches (Food company, Greece)**

- The proposed timeframe was feasible.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.

→ Application 7 was included in the phase II in-depth evaluation

**Application 8: Characterisation of antimicrobial extracts obtained from fermented agri-food by-products (University, Italy)**

- The proposed timeframe was feasible.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.

→ Application 8 was included in the phase II in-depth evaluation

After the Phase I pre-screening process, only three project applications (applications 6, 7 and 8) remained for the more comprehensive evaluation in Phase II. An overview of the nine criteria and the evaluation and rating process is provided in Table 2.

Following the Phase II in-depth evaluation, the three projects from application 6, 7 and 8 were selected as they met all necessary requirements and were deemed feasible for the service. All eight applicants were notified of the open call results and those whose applications did not pass received an email with a brief explanation as to why their project was not suitable for the service. Successful applicants were contacted to schedule meetings for further discussion and project refinement.



**Table 2** – Phase II in-depth evaluation criteria and scoring system from 1 = low to 5 = high for the three project applications that advanced from Phase I pre-screening.

|  | Application 6 -<br><b>Project 1</b>   | Application 7 -<br><b>Project 2</b>   | Application 8 -<br><b>Project 3</b>  |
|--|---|---|--|
| <b>Applicant</b>                               | SME   | Food company  | University   |
| <b>Country</b>                                 | Spain   | Greece  | Italy  |
| <b>Project title</b>                           | Proteomic and metabolomic characterization of the fermentation product <i>Bacillus subtilis natto</i> DG101 | Unravelling the microbial diversity and lipidome profile of Spanish-style and natural cv. Chalkidiki green table olives throughout fermentation and 12-month storage under low NaCl conditions at industrial scale: application of multi-omics approaches | Characterisation of antimicrobial extracts obtained from fermented agri-food by-products |
| <b>Previous experience</b>                     | 5   | 5   | 5  |
| <b>Selected service</b>                        | 5   | 3   | 3  |
| <b>Project relevance</b>                       | 5   | 4   | 4  |
| <b>Project objectives</b>                      | 4   | 4   | 4  |
| <b>Project timing</b>                          | 5   | 5   | 5  |
| <b>Project samples</b>                         | 5   | 5   | 5  |
| <b>Scientific relevance</b>                    | 4   | 4   | 4  |
| <b>Technological relevance and feasibility</b> | 4   | 4   | 4  |
| <b>Sustainability and economic benefit</b>     | 4   | 3   | 3  |
| <b>Total score</b>                             | 41/45   | 37/45   | 37/45  |



### **Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products**

The evaluation and rating process was carried out by the institute overseeing use case 2 (TUM) in collaboration with the institute responsible for conducting the majority of the technological experiments (IBA), which primarily assessed the technological relevance and feasibility of the applications. A total of five applications were submitted and eligible for the pre-screening. Below are the reasons for exclusion or inclusion from Phase II, based on the Phase I criteria:

#### **Application 1: The use of fermented goat whey in baking old variety grain bread and focaccia.** (Food business operator - SME, Italy)

- No schedule, time frame, or work plan was provided.
- Although compliant with METROFOOD-RI objectives and the open call, the logistical feasibility was restricted due to uncertainties regarding the microbial stability of the whey.
- The overall relevance was considered low due to the availability of existing literature on the topic and the absence of a novel scientific research approach.

→ Application 1 was rejected

#### **Application 2: Integration of multi-omics data for terroir analysis of different grape varieties grown in Northern Greece. The study includes the know-how of the producers, the winemaking practices and the overall footprint of their effect on the final product wine.** (Oenological Laboratory, Greece)

- The proposed timeframe of 3-6 months was not suitable for this use case.
- While compliant with METROFOOD-RI objectives, the proposal did not align with the use case specifications. The project aligns more with understanding local environmental impacts on wine quality, rather than developing new food products or ingredients from by-products, and further lacks the focus on circular bioeconomy principles or resource efficiency.
- The technology was not feasible for the partners involved in the project.
- This application was likely intended for use case 1 rather than use case 2.

→ Application 2 was rejected

#### **Application 3: New production systems of aquafaba ready-to-sell from chickpea waste for circular system. Organoleptic and chemical characterization of aquafaba.** (Food business operator - Industry, Italy)

- The proposed timeframe of 16 weeks was feasible.
- While compliant with the METROFOOD-RI objectives, the project did not align with the focus of use case 2, which centers on baked goods and novel ingredients.
- The technological approach was deemed unsuitable for the project partners.
- Scientific and technological excellence was lacking, as aquafaba products are already available on the market, and there is an abundance of published research on the subject.

→ Application 3 was rejected



**Application 4: Innovative integration of cereal by-products, spent black tea, and spent coffee in bakery products: enhancing ingredient design and process optimization for quality and shelf-life improvement** (University, Egypt)

- The proposed timeframe was feasible.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.
- The application from Egypt, a non-EU country, could be included based on the eligibility criteria allowing up to 20% of total TNA access for non-EU/non-associated projects.

→ Application 4 was included in the phase II in-depth evaluation

**Application 5: Investigation of effects of by-products generated at coffee bean roasting, and production of soya cheese and almond milk on quality and the health benefits of sourdough bread** (University, North Macedonia)

- The proposed timeframe was feasible.
- The proposal was compliant with the open call and overall METROFOOD-RI objectives.
- The technology was suitable for the project partners involved.
- Demonstrated solid scientific and technological excellence.
- The application from North Macedonia could be included based on the eligibility criteria that recognize both EU member states and associated countries. As an associated country, North Macedonia meets the requirements for participation alongside EU members, making it fully eligible for involvement in METROFOOD-RI. Additionally, North Macedonia is an integral part of the METROFOOD-RI consortium, contributing valuable expertise and resources that support the overarching goals of METROFOOD-RI.

→ Application 5 was included in the phase II in-depth evaluation

After the Phase I pre-screening process, only two applications (application 4 and 5) remained for the more comprehensive evaluation in Phase II. An overview of the nine criteria and the evaluation and rating process is provided in Table 3.

Following the Phase II in-depth evaluation, the two projects from application 4 and 5 were selected as they met all necessary requirements and were deemed feasible for the service. All five applicants were notified of the open call results and those whose applications did not pass received an email with a brief explanation as to why their project was not suitable for the service. Successful applicants were contacted to schedule meetings for further discussion and project refinement.



**Table 3** – Phase II in-depth evaluation criteria and scoring system from 1 = low to 5 = high for the two project applications that advanced from Phase I pre-screening.

|  | Application 4 -<br><b>Project 1</b>   | Application 5 -<br><b>Project 2</b>  |
|--|---|--|
| <b>Applicant</b>                               | Faculty of Agriculture, Ain Shams University  | Faculty of Technology and Metallurgy, Department of Food and Biotechnology, Ss Cyril and Methodius University  |
| <b>Country</b>                                 | Egypt   | North Macedonia  |
| <b>Project title</b>                           | Innovative integration of cereal by-products, spent black tea, and spent coffee in bakery products: enhancing ingredient design and process optimization for quality and shelf-life improvement | Investigation of effects of by-products generated at coffee bean roasting, and production of soya cheese and almond milk on quality and the health benefits of sourdough bread |
| <b>Previous experience</b>                     | 5   | 4  |
| <b>Selected service</b>                        | 2   | 5  |
| <b>Project relevance</b>                       | 4   | 5  |
| <b>Project objectives</b>                      | 3   | 4  |
| <b>Project timing</b>                          | 2   | 5  |
| <b>Project samples</b>                         | 5   | 5  |
| <b>Scientific relevance</b>                    | 4   | 4  |
| <b>Technological relevance and feasibility</b> | 4   | 3  |
| <b>Sustainability and economic benefit</b>     | 4   | 3  |
| <b>Total score</b>                             | 33/45   | 38/45  |



## 6. Description, planning and follow-up of the use case projects

Following the comprehensive evaluation process, the selected projects now move into the phase of detailed description, planning, and follow-up. This section focuses on outlining the structure and timeline of the chosen use cases, ensuring they align with the objectives of METROFOOD-RI. The next steps involve careful coordination and monitoring to ensure that the projects are successfully implemented and yield meaningful results. The planning stage addresses project logistics, and resource allocation, while follow-up ensures project execution, continuous assessment, and collaborative efforts across multiple institutes.

Owing to the similarity of the projects within each use case, the planning and follow-up for the projects within each use case are summarised together. However, where specific differences arise between the projects, the relevant details are highlighted accordingly.

### Characterisation of fermentation processes and their bio-based derived products from the food industries

#### Description & objectives

A total of three projects were selected by EUT as they perfectly fit within the focus of METROFOOD-RI use case 1. These projects are also well aligned with the expertise and capabilities of the service providers involved. All three projects share a common objective of using advanced scientific techniques to enhance the understanding and value of natural, fermented products for human health and functional food applications. Each project seeks to explore the bioactive compounds, microbiota, or metabolic profiles of specific fermented foods or by-products, aiming to optimize their nutritional benefits, safety, and marketability. Descriptions of each selected project are provided below, including their specific objectives. A visual representation of the different samples and experimental conditions to be tested is shown in Figure 3.

#### **Project 1: Proteomic and metabolomic characterization of the fermentation product *Bacillus subtilis natto* DG101**

This project focused on *Bacillus subtilis* DG101, a probiotic derived from the Japanese food NATTO, known for its cardiovascular, bone-strengthening, and anti-aging benefits (7, 8). NATTO, traditionally made from fermented soybeans, has limited acceptance in Europe due to its strong flavor and aroma. A Spanish company introduced a liquid probiotic that isolates the beneficial bacteria from NATTO, offering a more accessible alternative to European consumers. The project aimed to analyze the proteomic and targeted metabolomic composition of *Bacillus subtilis* DG101 in both pure fermentation cultures and the formulated probiotic, ready for consumption. This approach could provide a comprehensive understanding of the proteins and metabolites present in the probiotic, to better understand its health benefits and to help validate NATTO's potential as a functional food alternative in Europe.

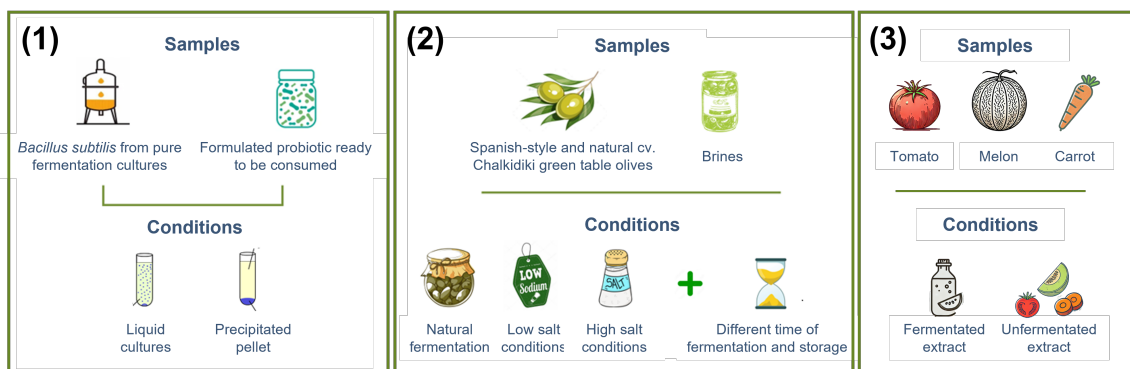
#### **Project 2: Unravelling the microbial diversity and lipidome profile of Spanish-style and natural cv. Chalkidiki green table olives throughout fermentation and 12-month storage under low NaCl conditions at industrial scale: application of multi-omics approaches**

Chalkidiki table olives make up about 50% of Greek production, with 92% being exported. They are mostly processed as Spanish-style green olives, which reduces health benefits and increases

salt content. In contrast, natural processing with minimal chemical intervention is more in line with consumer demand for minimally processed foods but poses challenges due to unpredictable fermentation (9). High salt content has also raised concerns about olives as a functional food. The company successfully fermented Chalkidiki olives in low NaCl brines, producing safe, accepted products and identifying microbial markers. The project aimed to explore low-salt fermentation methods, using advanced multi-omics approaches, including shotgun metagenomics and lipidomics, to explore correlations between microbiota, metabolites, and metabolic pathways. The goal was to improve product safety, quality, and nutritional value, supporting the development of table olives as a functional food rich in bioactive compounds.

### Project 3: Characterisation of antimicrobial extracts obtained from fermented agri-food by-products

Transforming high-impact waste-producing sectors like the food industry is essential, and can be achieved by promoting circularity, resource efficiency, and nature-based solutions to reduce food waste. Fermenting plant byproducts is a promising strategy, utilizing nutrients for microbial biomass or valuable metabolites. The research group focused on lactic acid fermentation of plant byproducts to create antimicrobial extracts for food processing. They have developed an extraction process for byproducts like melon, carrot, and tomato peels, producing effective antimicrobial compounds such as organic acids, peptides, and phenolics (10). This project aimed to chemically characterize these antimicrobial extracts and understand how metabolism modifies the matrix. The team could also assess safety, including potential contaminants. The results could support scientific dissemination and provide insights for effective technology transfer.



**Figure 3** - Visual representation of the tested samples and conditions in (1) Project 1, (2) Project 2 and (3) Project 3

### Users

Two food business operators and one university/research institute

### Use case providers

The service provision for the use cases involved a select group of institutes: EUT, IJZSRM, INSA, and TUBITAK. These partners were specifically identified in the Grant Agreement Description of Action (GA DoA) based on their specialised capabilities, aligning effectively with the requirements and offerings outlined in the call. The selection process was not open to all beneficiaries but limited to these pre-selected contributors, ensuring that the necessary technical expertise and resources were

readily available. The facilities were chosen for their high quality within the project's framework, validated methodologies, and streamlined collaboration potential, which facilitates efficient distribution and reduced operational effort. Table 4 provides a detailed overview of the tasks, experiments and analytical techniques and methodologies assigned to each partner institute within each project.

**Table 4** - Tasks and experiments provided by the respective partner institutes in use case 1.

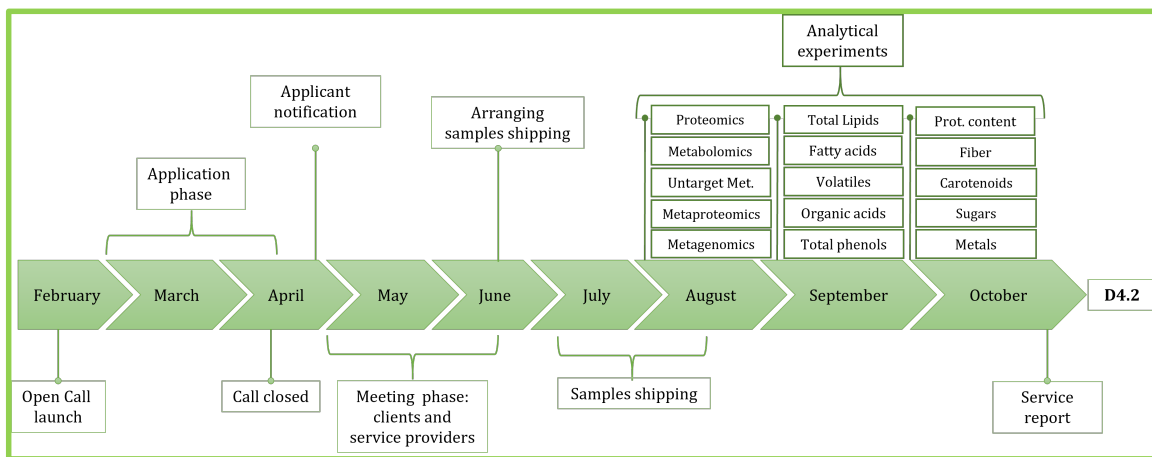
| Task/Experiments   | Analytical techniques & methodologies   | METROFOOD-RI Partner |           |           |
|--|---|----------------------|-----------|-----------|
|  |   | Project 1            | Project 2 | Project 3 |
| <b>Organization/Management</b>                             |   | EUT                  | EUT       | EUT       |
| <b>Metabolomic analysis</b>                                | GC-ESI-MS/MS, with a GC-QTOF  | EUT                  |           |           |
| <b>Untargeted metabolomic analysis</b>                     | LC-ESI-MS/MS, with a UHPLC-QTOF   |                      |           | EUT       |
| <b>Metaproteomic analysis</b>                              | nLC-ESI-MS/MS, with a nLC-Orbitrap  | EUT                  |           | EUT       |
| <b>Shotgun metagenomics</b>                                | NGS Illumina technology NextSeq 2000  |                      | EUT       |           |
| <b>Lipid composition</b>                                   | GC-FID  |                      | INSA      |           |
| <b>Fatty acid analysis on freeze-dried samples</b>         | GC-FID of FAME  |                      | INSA      |           |
| <b>Fatty acid analysis on fresh, vacuum sealed samples</b> | GC-FID of FAME  |                      | IJZRSM    | IJZRSM    |
| <b>Volatile profiling</b>                                  | GC-ESI-MS/MS, with a SPME-arrow and GC-QqQ  |                      |           | EUT       |
| <b>Organic acid analysis</b>                               | UV-Vis spectrophotry (Kit to determine the L-Malic acid UV method and Kit to determine the Citric acid UV method) |                      |           | INSA      |
| <b>Total phenol content</b>                                | UV-Vis spectrophotry (Falin-Cioucalteu)   |                      |           | INSA      |
| <b>Analysis of protein content</b>                         | AOAC 960.52 - Kjeldahl Digestion and titration by Tecator Kjeltch System 2300                                     |                      |           | TUBITAK   |
| <b>Analysis of total dietary fiber</b>                     | Vacuum system - FibreTech System cold extraction unit   |                      |           | TUBITAK   |
| <b>Analysis of carotenoids</b>                             | HPLC-UV   |                      |           | TUBITAK   |
| <b>Sugars</b>  | LIADe 122:2023   CLEN Method, Determination of Sugars in Food Products using HPLC                                 |                      |           | IJZRSM    |
| <b>Heavy metals and other minerals</b>                     | ICP-MS Plasma with microwave-assisted extraction  |                      |           | IJZRSM    |

After responsibilities were allocated, EUT coordinated communication with the applicants, organising meetings among the service providers, and managing the handling and shipping of samples. Additionally, EUT performed metabolomic and metaproteomic analysis and volatile profiling. INSA provided global fat analysis and a comprehensive fatty acid profile from freeze-dried samples, while, IJZRSM performed a fatty acid profile from fresh state, vacuum sealed samples. INSA also provided organic acid analysis and total phenol content measurements, TUBITAK was responsible for protein content, total dietary fiber and carotenoids analysis, while IJZRSM performed fatty acid profiles and determined sugars, heavy metals and other minerals. Throughout the project,

SCIENSANO, as WP4 task leader, was responsible for continuous monitoring, including risk management, reporting, and feedback analysis to ensure smooth service provision.

### **Planning and timeline**

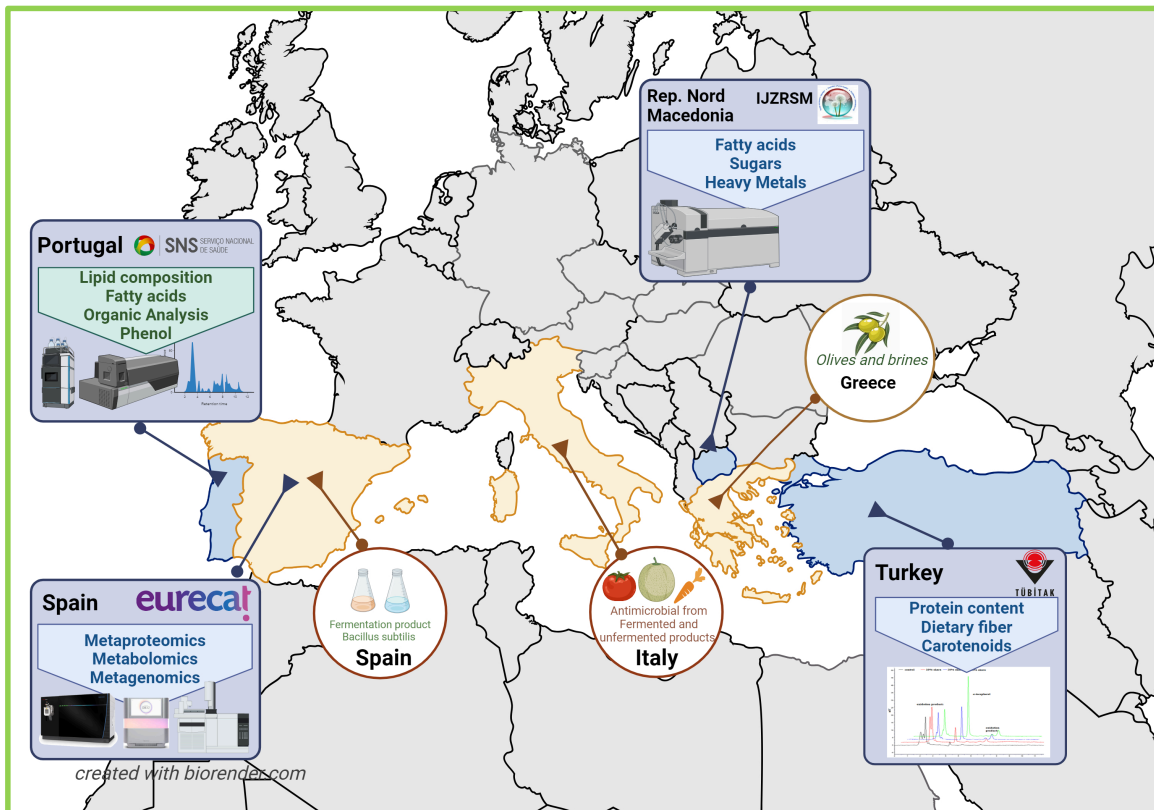
The open call launched on February 1<sup>st</sup> with the application phase running until April 20<sup>th</sup>. All applications were collectively evaluated, and users were notified of the results on April 30<sup>th</sup>. In May, the projects were further discussed and planned in detail with all participating institutes to assign analytical experiments based on their expertise. Shipping of raw materials from the users to EUT succeeded in June and July. The analytical procedures took place from August through October. Finally, a comprehensive report was prepared and sent to the users and a final meeting was scheduled to conclude the service provision. The entire process is visualized in Figure 4.



**Figure 4 - Detailed timeline for use case 1.**

### **Collaborative approach**

Active collaboration between the users and service providers was essential for the success of the use case projects within the proposed timeline. Effective communication and coordination among all involved parties ensured the smooth transfer of samples, analysis, and interpretation of data. Several initial e-mails were exchanged, and preliminary virtual meetings were held between each user and EUT to discuss the design, planning, and timelines. For projects 2 and 3, additional meetings were organised with all involved service providers to coordinate tasks and preempt potential issues, ensuring alignment among all partners. Following the completion of analyses, all relevant service providers shared their results and data through a centralised repository (SharePoint), used for storing data files such as sequencing results, lipidomic profiles, and reports. This ensured transparency, ease of access, and facilitated joint data analysis. A standardized reporting template was employed to ensure uniformity in data presentation, making it easier to integrate findings across different service providers. Finally, a detailed report was shared with all service providers and sent to the user. After the project's completion, a final meeting was organised to review the major outcomes, discuss the results, and explore potential future collaborations. The collaborative integrated approach is visualized within Figure 5.



**Figure 5** – Collaborative approach of partner institutes EUT (Spain), INSA (Portugal), IJZRS (North Macedonia) and TUBITAK (Turkey), visualising analytical partners (blue) and project applicants (orange).

### Difficulties/risks encountered

During the service provision of project 1, no risks or difficulties were encountered. This was likely due to the fact that only EUT was involved, eliminating the need for coordination between multiple service providers. Without an integrated service approach, processes were simpler and more direct, reducing potential delays and complications, allowing for smooth and efficient completion.

Although no issues arose in projects 2 and 3, careful planning helped mitigate several potential difficulties and risks. One common challenge was the logistics of ensuring timely and secure transportation of samples to the laboratories. Any delays or mishandling could have compromised sample quality, leading to inaccurate or inconsistent results. Fortunately, no such issues were encountered in any of the projects.

In project 2, the use of fresh versus freeze-dried samples across different laboratories could have introduced variability in lipidomic profiling results. Differences in sample preparation could affect lipid composition and fatty acid content, posing a risk to consistent findings. However, no significant variations were detected.



In project 3, a difficulty arose with detecting certain analytes due to their low concentration, which fell below the detection limits of the techniques used. As no additional or more concentrated samples were available, these analyses remained without quantifiable results, though they were still informative.

Lastly, coordinating efforts across multiple institutes with different schedules and laboratory processes could have posed a risk to the project's timeline. However, since there was no cascading workflow, each institute received samples directly from the user, avoiding dependencies and ensuring smooth progress.

### **Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products**

#### **Description & objectives**

A total of two projects successfully passed the pre-screening executed by TUM and IBA, as both aligned with the objectives of the open call and included feasible sub-projects within the allotted time frame. Both projects had a similar objective of including by-products from industrial food processing into bread as baking ingredients. Descriptions of each selected project are provided below, including their specific objectives. A visual representation of the different samples and experimental analysis is shown in Figure 6.

#### **Project 1: Inclusion of rice bran in wheat bread for preliminary experiments**

This project aimed to valorise cereal, tea, and coffee by-products by incorporating them into baked goods to enhance flavor, extend shelf life, and improve nutritional value. With expertise in by-product utilisation, the applicant wanted to develop innovative formulations that combine these ingredients, using advanced techniques like sensory analysis and chemometrics to optimise recipes. The project addressed the growing need for sustainable practices in the food industry, reducing food waste by repurposing underutilised resources rich in bioactive compounds, ultimately contributing to more sustainable food systems while offering economic benefits to SMEs and the wider food sector.

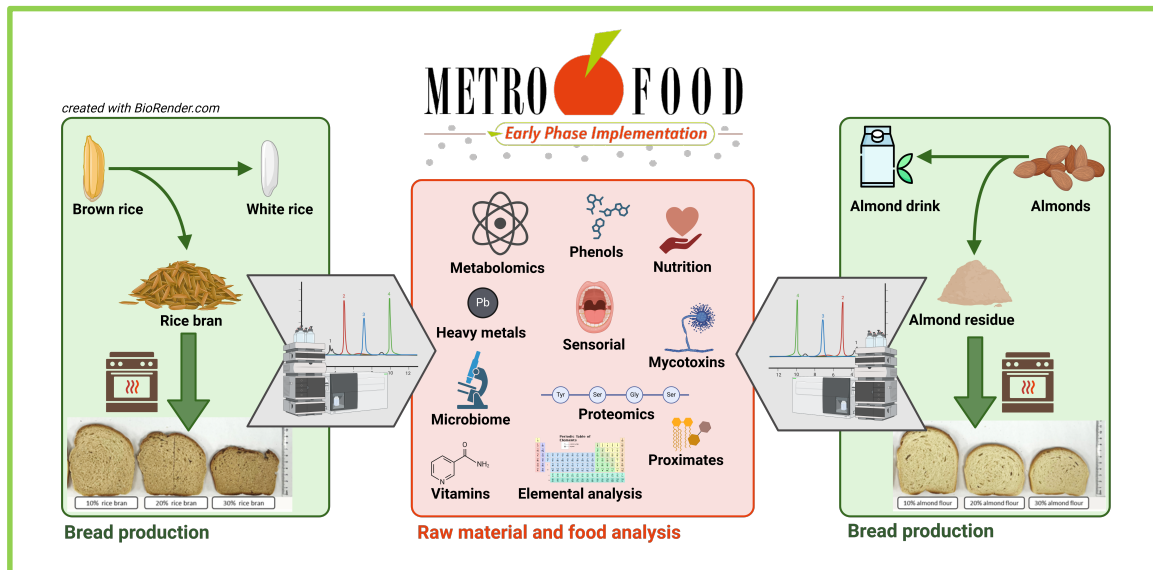
#### **Project 2: Incorporation of by-products from almond milk production into wheat bread**

This project explored the incorporation of coffee silverskin, okara, and almond by-products into sourdough bread to enhance its nutritional value, sensory qualities, and functional properties. Leveraging the applicant's extensive expertise in plant extracts and food by-products, the project aimed to valorise these underutilised resources while contributing to waste reduction and sustainability. Using advanced analytical techniques, the project could assess the optimal quantities of these by-products to improve bread formulations, aligning with circular economy principles and addressing the food industry's need for sustainable practices.

After discussion with the users and leading operators at TUM and IBA, the scope of both projects was significantly adjusted to fit within the tight time frame of the use case.

- For **project 1**, preliminary experiments were redirected to test the addition of rice bran to wheat bread, leveraging IBA's extensive experience with cereal by-products (11).

- For **project 2**, almond okara was selected as the focus for testing in wheat bread, due to its limited coverage in existing literature. The other by-products were excluded based on health concerns, with soy okara containing anti-nutritional properties (12) and coffee silverskin potentially exposing products to acrylamide or other heat-induced contaminants (13).



**Figure 6** - Visual representation of the samples and analysis of (1) project 1 and (2) project 2.

### Users

Two university/research institutes

### Use case providers

The following institutes were involved in the service provision for the use cases: TUM, IBA, AUTH, ENEA, CZU, and JSI. Similar to the selection rationale for use case 1, these partners were chosen based on the specialized capabilities outlined in the GA DoA. Each facility was selected specifically for its unique strengths and field of expertise, including advanced technical capabilities, established quality assurance processes, and validated methodologies that align closely with this use case's goals. The division of experiments between the service providers was of vital essence to meet the schedule requirements of the use case. The aminoacid analysis, for example, could have been executed by IBA but was transferred to CZU due to the high experimental load. Limiting the selection to these pre-identified contributors enabled the project to leverage streamlined collaboration with facilities that have robust distribution channels and the capacity to address the targeted requirements of the service provision with minimal additional effort. Table 5 provides a detailed overview of the tasks and experiments assigned to each partner institute.

**Table 5** - Tasks and experiments provided by the respective partner institute in use case 2.

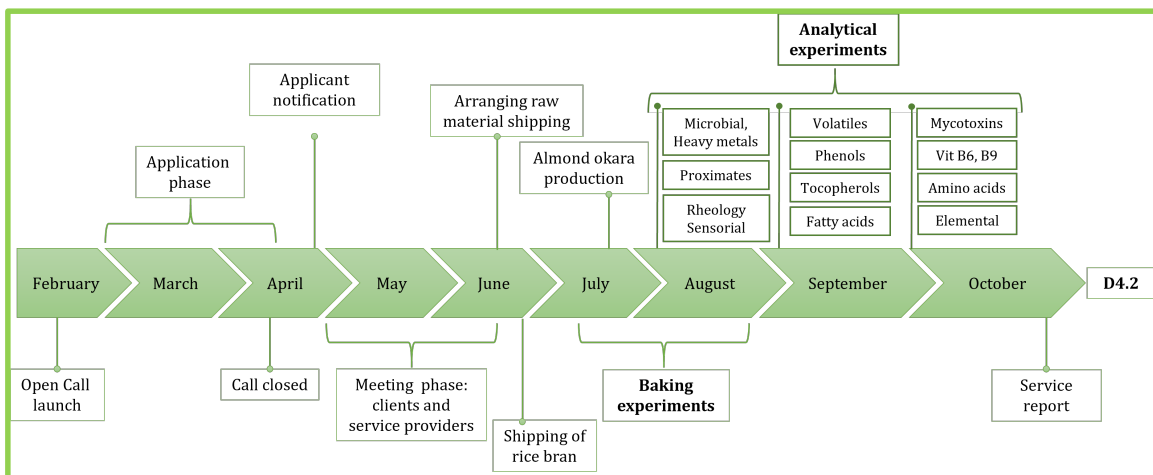
| Task/Experiments   | Analytical techniques & methodologies   | Partner |
|--|---|---------|
| <b>Organization/Management</b>   |   | TUM     |
| <b>Mycotoxins:</b> Alternaria, Fusarium, Aspergillus toxins  | SIDA LC-ESI-MS/MS   | TUM     |
| <b>Vitamins:</b> B6, B9  | SIDA LC-ESI-MS/MS   | TUM     |
| <b>Technology/Experimental plant</b>   | Pilot plant for bakery processing, manufacturing bread samples  | IBA     |
| <b>Proximates/Nutritional:</b><br>Water content<br>Crude protein<br>Crude ash<br>Crude fiber<br>Total fat,<br>Energy value | Thermobalance drying method (SR 90:2007) Kjeldahl-Kjeltec method (SR 13013-3:1994)<br>Calcining furnace<br>Fiber bag method (AOAC 991.43)<br>Soxhlet (SR 90:2007)<br>Calculation according to REG. EU 1169/2011 | IBA     |
| <b>Sensorial analysis</b>  | Trained sensory panel of 12 people assessing interior appearance, crumb flavor and the first bite.  | IBA     |
| <b>Safety for consumption:</b><br>microbial; heavy metals/trace elements<br>(Pb, Cd, Cr, Cu, Fe, Zn, Mn, Ni, Se)           | Total number of aerobic bacteria (ISO 4833-1/2014)<br>Total number of fungi (ISO 21527-2:2009),<br>Enterobacteriaceae (ISO 21528-2/2017);<br>ICP-MS with microwave-assisted extraction                          | IBA     |
| <b>Rheological testing</b>   | Dough Mixolab Analysis  | IBA     |
| <b>Fatty Acids</b>   | GC-FID of FAME  | AUTH    |
| <b>Bioactive compounds:</b><br>$\alpha$ -Tocopherol, $\gamma$ -oryzanol<br>Phenols   | HPLC-DAD-FL   | AUTH    |
| <b>Elemental analysis:</b> Ca, Cr, Cu, Fe, Mg, Mn, Ni, Zn, Al, Cd, Pb  | ICP-AES (2+3) with microwave-assisted mineralisation  | ENEA    |
| <b>Amino Acids</b>   | LC-ESI-MS/MS  | CZU     |
| <b>Volatiles</b>   | HS-SPME GC-MS   | JSI     |

After responsibilities were allocated, TUM coordinated communication with the applicants, organising meetings among the service providers, and managing the handling and shipping of samples. IBA, which hosts a pilot plant for bakery processing, led the technological aspect of the use cases by manufacturing the bread samples. Additionally, IBA conducted analyses on proximates, sensorial properties, nutritional content, rheological behavior, and microbial stability of the samples. Further analytical experiments were carried out by ENEA (elemental analysis), AUTH (polyphenol/fatty acids/tocopherols/oryzanol), JSI (volatiles), CZU (amino acids) and TUM (Vitamin B6, B9, B12 and mycotoxins). Throughout the project, SCIENSANO, as WP4 task leader, was responsible for continuous monitoring, including risk management, reporting, and feedback analysis to ensure smooth service provision.

### **Planning and timeline**

The open call was launched on February 1<sup>st</sup>, with the application phase running until April 20<sup>th</sup>. Before evaluating the submissions, TUM and IBA, as the main technological operators, held a feasibility assessment meeting to review the proposed projects. Applications were collectively evaluated, and users were informed of the results. In May, further discussions took place, involving users and staff from TUM and IBA's baking facility to refine project plans. After notifying applicants of the positive evaluations, detailed planning sessions were held with users, operators (IBA), and use case leaders

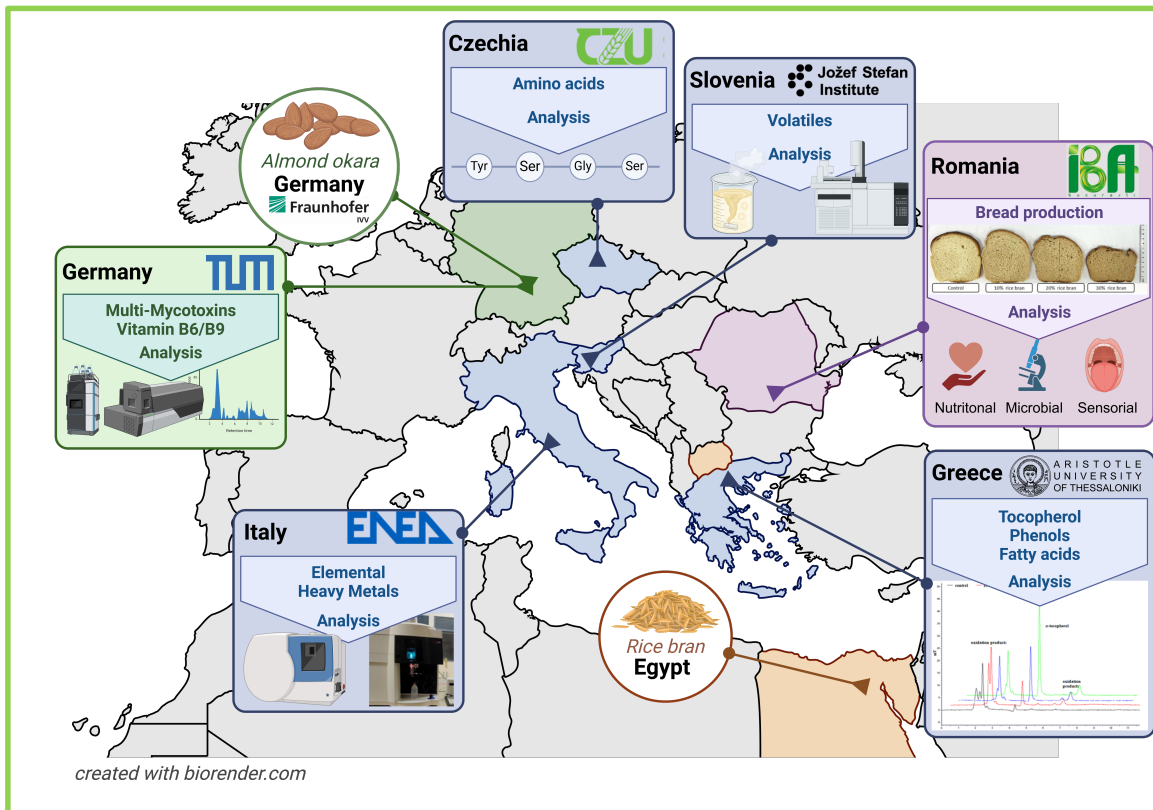
(TUM). Following project agreement finalization, all laboratory partners met to discuss the necessary analytical procedures and allocate experiments based on their expertise. In June and July, raw materials were shipped from the users to TUM, which acted as the central distributor, and then forwarded to the partner laboratories. Baking experiments were conducted at IBA from July through the end of August, while analytical procedures began in August and were finalised in October. The entire process is visualized in Figure 7.



**Figure 7 - Timeline for use case 2.**

### **Collaborative approach**

The planning and development of the use case project required active collaboration from the users to shape the research plan. This collaboration was based on the users' previous experience and the expertise of the main operators (IBA). Main communication with both users and service providers took place through email and virtual meetings. In individual meetings involving the users, main operators, and project management (TUM), the research objectives and project details for each case were defined. Once the project agreements were finalised, all laboratory partners involved in the use case convened to discuss the necessary analytical procedures and allocate experiments accordingly. The collaborative integrated approach is visualised within Figure 8.



**Figure 8** – Collaborative approach of partner institutes TUM (Germany), IBA (Romania), ENEA (Italy), CZU (Czechia), JSI (Slovenia) & AUTH (Greece) visualizing analytical partners (blue), country of organization/management, sample distribution and analysis (green), country of main execution (purple), project applicants (orange). North Macedonia (orange) provided the concept of the project while samples were provided by TUM (Germany).

### **Difficulties/risks encountered**

During the service provision, several risks and challenges emerged, primarily related to scheduling issues and legal complications involving the countries involved. First, differences in holiday schedules (such as Easter and Pentecost) between the participating countries and users caused communication delays during the first month of service provision in May. Additionally, legal challenges arose with customs and border controls when shipping rice bran samples from non-EU countries like Egypt and Saudi Arabia to Germany, resulting in prolonged customs clearance times.

Another issue was the inability to ship raw materials from North Macedonia to Germany, which required the almond okara to be produced in Germany to keep the project on track. This led to unexpected delays in the schedule due to the need for almond okara production. Further disruptions occurred when some analytical instruments were damaged, and certain laboratories were under reconstruction, causing delays in the timeline or necessitating the use of alternative analytical methods. Finally, compliance with the Nagoya Protocol, which ensures the fair and equitable sharing of benefits from the use of genetic resources, posed another challenge (14). If the research data were to be published, it would require an application to the appropriate Nagoya office in the country where



the raw materials were cultivated. For example, the almonds used in project 2 were harvested in Valencia, Spain, requiring permission from the Spanish Nagoya office - a process that could take up to six months.

## 7. Discussion

This section outlines the challenges encountered during the actual service provision for the different projects, the strategies employed to mitigate risks, and insights gained for improving future service provision. The key issues revolved around sourcing raw materials, technical limitations, compliance with international protocols, logistical delays, etc. Reflecting on these challenges offers valuable lessons for streamlining future collaborations and ensuring smoother execution not just of similar projects, but more widely of the infrastructure's service provision in the next future.

### Characterisation of fermentation processes and their bio-based derived products from the food industries

#### Risks and mitigation measures

Across the three projects, several risks were identified, each with corresponding mitigation strategies to ensure the smooth progression of the work:

- **Probiotic stability (project 1):** The risk of *Bacillus subtilis* DG101 degradation during sample preparation or transport could have affected the accuracy of proteomics and metabolomics analyses. To mitigate this, probiotic samples were carefully handled and stored under conditions that preserved bacterial integrity, minimising the risk of instability.
- **Data inconsistencies or misinterpretation (project 1):** There was a risk of inconsistencies in proteomics and metabolomics data, potentially leading to misinterpretation. This was mitigated through extensive data validation using robust software tools such as Proteome Discoverer and MASCOT to ensure accurate and reliable results.
- **Low NaCl conditions and pathogen growth (project 2):** The use of low-salt brines during olive fermentation posed a risk of pathogen and spoilage organism growth due to reduced salinity levels. To address this, proper preservation methods, such as vacuum sealing and controlled storage, were employed to maintain the safety and quality of the samples during transport and processing.
- **Lipid profile variability (project 2):** Differences in sample preparation and handling could have introduced variability in lipid profiles across laboratories. To mitigate this, lipid analysis was performed using both fresh and freeze-dried samples across two labs. In future work, cross-validation and interlaboratory methods can be applied to further reduce variability.
- **Logistical challenges (project 3):** The secure and timely transport of samples was identified as a potential risk, with delays or mishandling possibly compromising sample integrity. This risk was mitigated through careful planning, clear communication with users, and proper labeling, ensuring smooth and safe sample transport.
- **Detection limit of analytes (project 3):** Some analytes were present in concentrations below the detection limits of the techniques used. While more sensitive methods or concentrated samples would be a future solution, in this project, the lack of additional samples prevented further analysis, and this limitation was noted.

- **Coordination across institutes (project 2 & 3):** Coordinating efforts across multiple institutes, each with different schedules and processes, posed a risk to the project's timeline. This was mitigated by avoiding a cascade work design, allowing institutes to work independently without dependencies, leading to smoother and more efficient coordination.

### **Future prospects**

The future prospects of these projects highlight the potential for continued research, market expansion, and industrial applications across various fields.

- ***Bacillus subtilis* DG101 and market expansion (project 1):** The project has laid a strong foundation for further research into the health benefits of *Bacillus subtilis* DG101, potentially leading to new clinical studies that link its proteome and metabolite profiles to additional outcomes like anti-aging and cardiovascular protection. With the probiotic addressing the sensory challenges of traditional NATTO, there is significant potential for market expansion in Europe. The detailed characterization of bioactive compounds could support the marketing of this probiotic as a scientifically backed dietary supplement.
- **Advancements in table olive production (project 2):** This study offers promising prospects for transforming the production of healthier, low-sodium table olives. By applying a multi-omics approach, the project reveals how fermentation conditions influence microbial and lipid profiles, which could lead to improved nutritional properties and functional food status for table olives. Future research could explore how microbial bacteria and lipid biomarkers identified in this project can further enhance the health benefits of table olives, aligning with growing consumer preferences for minimally
- **Fermentation of agri-food by-products (project 3):** The project has provided valuable insights into the antimicrobial properties of fermented agri-food by-products, opening up new possibilities for both research and industrial applications. These findings may lead to the development of natural preservatives for the food industry or antimicrobial agents for pharmaceutical applications. Further research could focus on optimising fermentation processes, scaling up production, and exploring other by-products as potential raw materials. There are also opportunities for collaboration with food industries for technology transfer or with policymakers to promote waste valorisation, supporting the transition to a circular economy.

### **Lessons learned**

The lessons learned from the three projects highlight several key aspects that contributed to their success. First, clarity in defining objectives was crucial. Well-defined goals enabled the team to execute analytical approaches smoothly and with a clear focus. Similarly, conducting all analyses within a single facility, where possible, simplified operations, and helped avoid the delays and risks that are often associated with multi-center collaborations.

Proactive risk management played a significant role across all phases of the projects. While no major difficulties arose, regular monitoring, clear planning, and continuous communication ensured that the projects remained on track. These practices were vital for anticipating and addressing any potential challenges, particularly in coordinating efforts across multiple institutions. Another key lesson was the importance of having a clear point of contact. Each service provider had a designated



coordinator, which greatly improved communication and task management. For instance, EUT took charge of general communication, while INSA and IJZRSM managed lab results and logistics, ensuring smooth collaboration.

Methodological flexibility emerged as another essential factor. The ability to adapt to different sample processing methods, such as comparing fresh versus freeze-dried samples, provided a more comprehensive understanding of how sample handling affects lipid and fatty acid profiles. This adaptability also extended to dealing with issues like low analyte concentrations, where a flexible approach proved invaluable in maintaining research continuity.

Projects 2 and 3 also underscored the value of collaboration among multidisciplinary teams. The complexity of olive fermentation, for example, required input from microbiology, next-generation sequencing, lipidomics, and fermentation processes, illustrating how the integration of various fields can lead to more robust research outcomes. Strong collaboration between research institutes with distinct areas of expertise significantly enhanced the quality of the research. Clear communication frameworks and coordination mechanisms helped overcome logistical challenges and ensured data consistency across all partners.

### **Project conclusions**

The projects showcased successful collaborations and efficient workflows that set a strong foundation for subsequent research. Collaboration among the laboratories proved to be highly effective, fostering a productive exchange of expertise and resources. Sample distribution was well-coordinated, enabling timely analysis across different facilities and minimizing logistical delays. Participating labs followed a harmonised framework for data sharing, which significantly contributed to reliable and comparable results. The collective effort ensured that risk mitigation strategies, including probiotic stability and lipid profile variability, were implemented seamlessly, delivering robust outcomes and demonstrating the value of shared methodologies. The projects also highlighted several critical areas for future focus and improvement. While the mitigation strategies for risks such as probiotic stability and lipid profile variability were effective, future projects should implement more standardised protocols across all participating laboratories to minimise variability and ensure higher reproducibility. Establishing stronger interlaboratory validation processes and cross-checking methods could further enhance data consistency. Additionally, the research into low-sodium olive fermentation revealed a need for more in-depth studies on microbial dynamics and lipidomics to optimise both nutritional benefits and flavor profiles. Scaling up these fermentation processes will require fine-tuning the balance between microbial diversity and salinity levels, while also expanding the scope of lipidomics to include a more detailed analysis of polar lipids and fatty acid profiles. For agrifood by-product fermentation, further exploration is needed to optimise the antimicrobial properties of these by-products, particularly with a focus on scaling the production processes and enhancing commercial viability. A critical next step will involve developing stronger partnerships with the food industry for technology transfer, as well as engaging policymakers to support regulatory frameworks that promote waste valorization in line with circular economy principles.



## **Pilots for food production and integrated analytical services: development of novel products with ingredients including alternative proteins and/or food-by-products**

### **Risks and mitigation measures**

One of the major challenges in this project was obtaining raw materials from the users. For the rice bran shipment from Egypt via courier service, extensive documentation and bureaucratic measures were necessary to import the rice bran, leading to a delay of approximately two weeks. On the other hand, the user from North Macedonia faced issues with shipping raw materials to Germany, making it impossible to send the required almond okara. To resolve this, TUM collaborated with Fraunhofer IVV to supply the almond okara, thus accelerating the project timeline. For future projects, it is essential that the supply of raw materials be guaranteed by the user or discussed with the RI in advance. Additionally, for sensitive samples requiring refrigerated transport, pre-arranged solutions should be in place. Challenges related to customs and the stability of samples should also be addressed before entering a service provision contract.

The Nagoya Protocol posed another obstacle for this project, and it must be taken into account for future research endeavors. One approach could be to use materials exclusively from non-Nagoya countries, though this may not be practical, particularly when applicants from Nagoya countries wish to provide their own materials. To mitigate this, a clear agreement should be established before service provision begins, ideally with the involvement of legal experts from the RI. Key questions must be resolved in advance, such as whether the research data will be published by the RI or the user, and who will be responsible for compliance with the Nagoya Protocol.

### **Future prospects**

Building on the success of the service, there is significant potential to drive innovation in food production, expand market opportunities, and further integrate sustainable practices within the food industry. These initiatives not only address the growing consumer demand for healthier, enriched food products but also promote the utilization of alternative proteins and food by-products, contributing to a more circular economy.

- **Enrichment of baked goods and processed foods (project 1 & 2):** The successful application of positive compound enrichment from side-streams offers promising avenues for expanding this approach to a wider range of food products, including baked goods, processed foods, and cereal-based products such as pasta. Moreover, the fortified products received positive feedback in sensory evaluations, suggesting strong potential for market acceptance. Future studies could focus on optimizing enrichment methods and exploring new side-stream sources to maximize the health benefits and sustainability of these products.
- **Enrichment with health-promoting compounds using rice bran (project 1):** Rice bran has demonstrated significant potential for enrichment with health-promoting compounds such as vitamins,  $\gamma$ -Oryzanol, phenols (*p*-coumaric and ferulic acids) or unsaturated fatty acids, making it a valuable ingredient for enhancing the nutritional profile of various food products. For instance, the vitamin B6 content that rice bran adds to bread qualifies the product to carry a "source of vitamin B6" health claim under Regulation (EC) No 1924/2006. Future research could identify additional bioactive compounds in rice bran that provide health benefits, potentially opening new market opportunities for vitamin-fortified foods.
- **Integration of almond okara into food products (project 2):** Almond okara presents possibilities for the development of novel food products with enhanced nutritional value.



Future research could focus on optimizing the processing of almond okara for large-scale food production and exploring its use in other food categories, aligning with efforts to reduce food waste and promote sustainability.

### **Lessons learned**

To ensure the smooth execution of future projects, it is recommended that all institutes involved provide cost estimates upfront and handle the purchase of consumables and chemicals themselves. While this approach aligns with the intended process, it was not feasible under the current financial management of the METROFOOD-EPI project. This process should be streamlined once the RI is fully operational. In this particular use case, the experiments were carried out by a predetermined group of laboratories as outlined in the project proposal. For future service provisions, the availability and feasibility of RI facilities should be confirmed before finalising project agreements. Furthermore, delays caused by differing holiday schedules across countries were a notable issue, though this can be mitigated through careful planning and by setting realistic timeframes, which proved challenging in this use case.

### **Project conclusions**

A key challenge in the project was the difficulty in obtaining raw materials from the applicants, which led to delays. To avoid such issues in future projects, ensuring raw material supply in advance and addressing customs and sample stability requirements before project initiation is essential. Additionally, sensitive samples should have pre-arranged transport solutions. The Nagoya Protocol also posed complications, highlighting the need for early legal guidance and clear agreements on compliance responsibilities and data publication. Future projects should consider sourcing materials from non-Nagoya countries where feasible, and legal experts should be involved early on. Despite these challenges, the collaboration among service providers was exemplary. Tasks were shared effectively, maximising efficiency across teams and ensuring that each service provider could leverage their expertise to support project goals. This collaborative approach facilitated on-time delivery of results, demonstrating the RI's capacity to coordinate complex workflows. For even smoother execution, it's important to establish cost estimates upfront and streamline the procurement process. Coordination among participating institutes should be confirmed early, and realistic timelines should account for differing holiday schedules. Pre-emptive planning and clear communication will be vital in mitigating these risks going forward.



## 8. User engagement and satisfaction

### 8.1 User engagement

For the transnational access services, the announcement of the respective open call was published on the dedicated webpage (<https://www.metrofood.eu/access/open-call.html>) and featured as the main news on the homepage at the time of the call launch. The same announcements were also shared on METROFOOD-RI's social media platforms and the official websites of several consortium members. Additionally, potentially interested users were contacted directly via emails through mailing lists of the consortium partners, leveraging their professional networks. To facilitate communication and support regarding the open calls, a dedicated email address ([calls@metrofood.eu](mailto:calls@metrofood.eu)) was provided and shared across all communication channels used for the announcements.

### 8.2 User satisfaction

User satisfaction is critical to the success and long-term sustainability of METROFOOD-RI, as it helps gauge the effectiveness of the RI's services from the perspective of its end-users. By actively gathering feedback, METROFOOD-RI can identify strengths and areas for improvement, ensuring that services remain relevant, efficient, and aligned with user needs. High satisfaction levels also foster stronger relationships with stakeholders, including researchers, industry partners, and policymakers, promoting trust and encouraging further collaboration. Moreover, user feedback provides valuable insights for refining access procedures and operational workflows, contributing to continuous improvement and innovation within the RI. Therefore, user satisfaction surveys for the provided use case services have been designed to evaluate satisfaction across various functional aspects. The survey, created using LimeSurvey, were distributed to users after the service results were communicated, ensuring users have complete information before providing feedback. The actual survey results will not be included in the deliverable, as they are primarily intended for internal evaluation to support continuous improvement and service optimisation. This allows METROFOOD-RI to thoroughly assess and analyse the feedback before making any adjustments to the services. An overview of the survey questions can be found in Annex I.

### 8.3 Socio-economic impact

The socio-economic impact of METROFOOD-RI is particularly significant due to its focus on promoting sustainability, innovation, and collaboration in the food and nutrition sectors. One key area of impact is METROFOOD-RI's role in advancing sustainable food production methods. By supporting the valorisation of by-products from food processing and encouraging the use of alternative, nutrient-rich ingredients, METROFOOD-RI helps industries reduce waste and improve resource efficiency. This shift towards a circular economy not only minimises the environmental footprint of food production but also opens up new market opportunities for innovative, sustainable products. These developments strengthen the competitiveness of the European food sector, fostering its leadership in global markets.

In addition to promoting sustainability, METROFOOD-RI enhances food safety and traceability. By providing access to cutting-edge analytical services and scientific methods, it enables food businesses to improve product quality and meet stringent safety standards. This reduces the risk of foodborne illnesses, lowers the economic burden associated with product recalls, and boosts consumer



confidence. The RI also plays a critical role in supporting global trade by helping industries comply with international food safety regulations, which is essential for maintaining a strong position in competitive markets.

The TNA services further amplify its socio-economic impact by facilitating collaboration between research institutions, industries, and policymakers. This integrated approach not only drives technological innovation but also helps shape food safety regulations and public health policies. The resulting improvements in food standards contribute to long-term socio-economic benefits, such as reduced healthcare costs, enhanced public health outcomes, and better nutrition standards.

Moreover, METROFOOD-RI contributes to economic growth by fostering innovation and improving supply chain efficiency. By enabling food-related industries to adopt new technologies and practices, it supports the development of high-value products and services. This boost to the knowledge-based economy is particularly beneficial for SMEs, which play a critical role in driving innovation and economic diversification.

On a global scale, METROFOOD-RI's efforts to improve food safety and sustainability extend beyond Europe. The infrastructure helps countries with less advanced food safety systems adopt higher standards, promoting more equitable trade relationships and enhancing food security. This global engagement ensures that innovations in food safety and sustainability benefit a wider range of stakeholders, contributing to improved public health and more resilient food systems worldwide.

Finally, METROFOOD-RI's contributions to education and training help prepare the next generation of scientists, technologists, and policy experts. By providing access to advanced research tools and fostering international collaboration, the infrastructure supports the continuous development of expertise in food safety, sustainability, and resource management, ensuring a lasting impact on both the scientific community and the food industry.



## 9. Conclusions and plans for the next steps

Looking ahead, the next steps for METROFOOD-RI involve scaling its services and integrating its physical and electronic components into a seamless system capable of serving a broader and more diverse user base. The success of the transnational access services has laid a strong foundation for expanding the infrastructure's reach, accommodating more complex, high-priority applications, and increasing participation from institutions across various countries. This expansion reflects the foundational work established in defining core specifications, access procedures, and service updates, which have provided crucial insights into service provision and operational specifications. The use cases further provided valuable insights for refining these procedures, ensuring that METROFOOD-RI's service provision, from request to delivery, aligns with its excellence-driven and market-focused goals. Moreover, lessons learned have highlighted the need for improving communication frameworks and setting clearer timelines across institutions, as well as ensuring the availability of raw materials and addressing logistical challenges proactively.

By focusing on deepening engagement with industry, particularly through partnerships with food businesses and SMEs, METROFOOD-RI can further drive the sharing, transfer and exploitation of its innovations. This will not only accelerate technology transfer but also stimulate economic growth and promote the adoption of sustainable practices throughout the food sector, as outlined in the strategic objectives of WP5 focused on roadmapping, impact and long-term sustainability. On a global scale, METROFOOD-RI has the potential to shape international food safety and sustainability policies by providing the necessary scientific data to harmonise standards. Engaging with bodies like the European Food Safety Authority (EFSA) and the United Nations, the infrastructure can play a pivotal role in creating a more unified regulatory environment, benefiting both producers and consumers. Additionally, METROFOOD-RI's educational and capacity-building programmes will be essential for training the next generation of experts in food safety, sustainability, and metrology, ensuring long-term progress in these critical areas, which is a focus point METROFOOD-EPI WP6 on dissemination, outreach, and community engagement.

As the infrastructure continues to grow, promoting circular economy principles and developing scalable models for resource efficiency in food production will remain central to its mission. Through these advancements, METROFOOD-RI will continue to enhance its socio-economic impact, securing its position as a key player in the future of global food systems.



## List of abbreviations

CH - Central Hub  
DoA - Description of Action  
EC - European Commission  
EFSA - European Food Safety Authority  
e-RI - electronic-RI  
FBO - Food business operators  
FAME - Fatty acid methyl ester  
GA - Grant Agreement  
GC-FID - Gas chromatography with flame ionisation detector  
GC-MS - gas chromatography-mass spectrometry  
GC-QTOF - gas chromatography-quadrupole time of fly mass spectrometry  
GC-QqQ - gas chromatography-triple quadrupole mass spectrometry  
HPLC-DAD-FL - High pressure liquid chromatography with diode array/flourimetric detection  
HS-SPME - head space-solid-phase microextraction  
ICP-AES - Inductively coupled plasma atomic emission spectroscopy  
ICP-MS- Inductively coupled plasma ionisation mass spectrometry  
LC-ESI-MS/MS Liquid chromatography with electro-spray ionisation tandem mass spectrometry  
METROFOOD-PP - METROFOOD Preparatory Phase  
NGS - Next Generation Sequencing  
nLC-ESI-MS/MS nano Liquid chromatography with electro-spray ionisation tandem mass spectrometry  
NN - National Nodes  
P-RI - Physical-RI  
RI - Research Infrastructure  
RM - Reference Material  
SIDA - Stable isotope dilution analysis  
SME - Small to medium-sized enterprises  
TNA - Transnational Access  
UHPLC—Ultra High pressure liquid chromatography  
UV-Vis - Ultraviolet Visible  
WP - Work Package



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## Annex I - User Satisfaction Survey

\*Please indicate the type of your organization:

- Commercial Laboratory or Research Facility
- Public Research Institute / University
- Food Production/Packaging/Processing Business
- Authority / Policy maker / Food Inspection or Control Agency
- Consumers/Citizens (incl. associations and schools)
- Organization
- Other:

\*Please indicate your own position within your organization:

- Senior Investigator
- Junior Investigator
- (PhD) Student
- Nutrition Specialist
- Higher Management
- Middle Management
- Lab Technician
- Quality Control
- Other:

\*Please rate the following aspects of the service provision of METROFOOD-RI:

|                                    | Application procedure and registration | Response time of METROFOOD-RI | Communication and User support | Technical support (e.g. on sample preparation, transport, etc.) | Report quality        | Reporting time        |
|------------------------------------|--|-------------------------------|--------------------------------|---|-----------------------|-----------------------|
| Very dissatisfied                  | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |
| Dissatisfied                       | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |
| Neither satisfied nor dissatisfied | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |
| Satisfied                          | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |
| Very satisfied                     | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |
| Not applicable                     | <input type="radio"/>                  | <input type="radio"/>         | <input type="radio"/>          | <input type="radio"/>   | <input type="radio"/> | <input type="radio"/> |



\*How would you rate your overall experience as a user of this METROFOOD service?



\*Based on your present experience, how likely are you to recommend METROFOOD as a service provider for this type of services?



\*Do you have any comments or additional feedback on the previous questions in this survey?

\*Do you have any suggestions to improve this service provision?