agriloop

high-value products from agricultural residues through sustainable chains

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DEM	Demonstrator, Pilot, Prototype	☐
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1. Executive Summary

This document provides the second batch of Practice Abstracts (PAs) for the AgriLoop project. The work's core objective, carried out under Work Package 5 (WP5), is to translate the project's specific scientific and technical outcomes into clear, actionable recommendations intended for various stakeholders, including farmers, industry professionals, and the research community. They offer focused, practical insights, ensuring the generated knowledge is accessible and ready for real-world application.

Collection methodology and standardisation

We worked directly with project partners from across Europe and China to gather the most relevant project results, relying on their expertise as they are the primary experts on their specific work streams. However, to aid this process Work Package 5 (WP5) created a standardised approach to ensure quality and coherence across all contributions (see annex 1). This included:

1. **Production of a comprehensive guide:** A detailed guide was distributed to all contributing partners, outlining the specific objectives, target audience, and required tone for the BPAs.

2. **Provision of a template and examples:** A clear template was provided to streamline content creation, along with concrete examples from D5.8 abstracts and other similar European projects to illustrate best practices in effective knowledge transfer.

This approach ensured consistency and reduced the burden on partners. Additionally, for maximum readability and ease of information extraction, all abstracts have been organised into three distinct, structured sections: an introduction (contextualising the problem and residue); **Project activity** (detailing the methods and execution); and **Key findings and recommendations** (presenting the results and actionable steps).

Thematic summary of batch 2 abstracts

The 13 abstracts in this batch collectively highlight significant advancements in the sustainable processing of agricultural waste. The core themes revolve around:

- **Valorisation of diverse residues:** Focusing on using residues from various agricultural processes—specifically peanut red skin, potato peels, tomato pomace, and grey starch—to unlock their value in the production of high-demand products like biopolymers and alternative proteins.

- **Sustainable and green extraction technologies:** Demonstrating the effective application of innovative, environmentally friendly processing methods, notably the use of Natural Deep Eutectic Solvents (NADES) and ultrasonic-assisted extraction techniques, to replace conventional, less sustainable solvents.

- **Characterisation of bioactive compounds:** Concentrating on identifying, quantifying, and optimising the recovery of high-value functional compounds, such as polyphenols (proanthocyanidins, resveratrol), for potential use in the food, feed, and cosmetic industries.

- **Digitalisation and software development:** Highlighting the role of digital tools, including software development for data processing and analysis, to improve efficiency, traceability, and the management of complex biorefinery processes.

- **Environmental impact and policy:** Drawing attention to findings related to the environmental performance of processing techniques, providing a basis for developing informed policy recommendations for sustainable waste management and circular economy regulations.

The results presented in this batch confirm the technical feasibility of several processes and biorefinery concepts, moving them closer to industrial readiness.

2. Practice abstracts

2.1 Extraction efficiency of polyphenols from peanut red skin

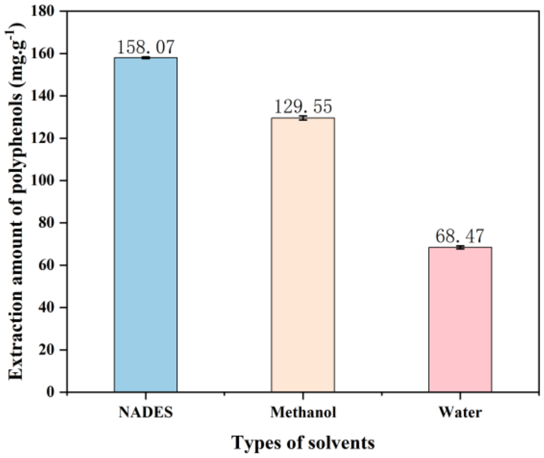
Contributors: Aimin Shi (IFST-CAAS), Hui Hu (IFST-CAAS)

Peanut is a key oil crop in China and its seed coat (peanut red skin) is an important by-product of peanut processing. Each year, more than 400,000 tons of peanut red skin are generated in China, yet its valorisation remains limited. Notably, peanut red skin is rich in bioactive phenolic compounds, including proanthocyanidins and resveratrol, with high nutritional and functional value. Natural deep eutectic solvents (NADES) have emerged as promising, environmentally friendly solvents for extracting such compounds.

NADES are formed by mixing hydrogen bond donors and acceptors in defined molar ratios, offering easier preparation and biodegradability. Their dense hydrogen-bonding network efficiently extracts polyphenols while preserving structural integrity. They also provide enhanced safety and lower flammability versus conventional solvents like ethanol, reducing infrastructure costs. As such, using NADES for the extraction of polyphenols and other components from peanut red skin could enable high value uses for peanut processing waste.

Project activity: Ultrasonic-assisted extraction of polyphenols from peanut red skin was carried out using NADES in comparison with conventional solvents (methanol, ethanol, and water). Eight NADES formulations were prepared, with choline chloride as the hydrogen bond acceptor (HBA) and ethylene glycol, 1,2-propanediol, glycerol, lactic acid, citric acid, urea, glucose, and sucrose as the hydrogen bond donors (HBDs). Extraction efficiency was assessed by varying key parameters, including HBA/HBD molar ratio, solid-to-solvent ratio, water content in NADES, and ultrasonic conditions (time, temperature, power). Single-factor experiments were conducted by varying each parameter within the following ranges: extraction time (5–30 min), liquid-to-solid ratio (10–50 mL/g), choline chloride/lactic acid molar ratio (3:1 to 1:3), ultrasonic power (200–400 W), temperature (30–70 °C), and water content (10–50%). The process is currently being evaluated at pilot-scale.

Key findings and recommendations and recommendations: The optimal extraction conditions were identified as: choline chloride:lactic acid (1:1 molar ratio), 30% water content, liquid-to-solid ratio of 10 mL/g, ultrasonic power of 250 W, ultrasonic temperature of 40℃, and ultrasonic time of 20 min.



Under these conditions, the polyphenols yield reached 158.07 mg/g, representing a 2.3-fold increase over water extraction and 1.2-fold increase over alcohol extraction. In addition, polyphenols were recovered by static adsorption-desorption using macroporous resin, followed by freeze-drying and the resulting extracts exhibited strong in-vitro antioxidant activity.

References:

- **Novel solvent properties of choline chloride/urea mixtures.** Chem Commun (Camb)(1), 70-71. Abbott, A. P., Capper, G., Davies, D. L., Rasheed, R. K., & Tambyrajah, V. (2003). https://doi.org/10.1039/b210714g
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- **Enzyme-assisted supercritical fluid extraction: An integral approach to extract bioactive compounds.** Trends in Food Science & Technology,

116, 357-369. Patil, P. D., Patil, S. P., Kelkar, R. K., Patil, N. P., Pise, P. V., & Nadar, S. S. (2021). https://doi.org/10.1016/j.tifs.2021.07.032

- **Green and sustainable solvents of the future: Deep eutectic solvents.** Journal of Molecular Liquids, 379, 121676. Prabhune, A., & Dey, R. (2023). https://doi.org/10.1016/j.molliq.2023.121676

2.2 Valorization of wet apple pomace for pectin extraction

Contributor: Xin Jin (IFST-CAAS)

Pectin is valued by the food industry as a natural source of thickeners, stabilizers and emulsifiers and has applications in the pharmaceutical and cosmetic industries. Apple pomace is the second-largest source of commercial pectin production but remains underutilized due to the environmental and economic drawbacks of conventional acid extraction methods.

Green extraction technologies have the potential to improve the efficiency of pectin production from apple pomace, reducing extraction time and improving yields. This study explores the application of such approaches to dry and wet apple pomace from SDIC Zhonglu Fruit Juice Co., Ltd.

Project activity: For dry apple pomace, ultrasonic-assisted extraction was combined with deep eutectic solvents (UAE-DES). A single-factor and response surface methodology was used to optimise the process. The optimal conditions were identified as: choline chloride: citric acid (1:2 molar ratio), 30% water content (w/w), liquid–solid ratio of 40 mL/g, extraction at 81°C for 40 min, and ultrasonic power intensity of 25%. Under these conditions, a pectin yield of 25.06 ± 0.87%* was obtained (*amount of mass recovered as a % of original product). DES recyclability is currently under investigation.

For wet extraction, fresh undried pomace (75–80% moisture) was processed using enzymatic treatments (cellulase and xylanase) combined with physical methods (ultrasound or microwave). This approach yielded up to 20% pectin, an improvement over physical treatments alone, which yielded around 16%. Moreover, wet extraction, using undried pomace, avoids energy-intensive drying.

Key findings and recommendations: These approaches show promise for biorefineries and food processors seeking more efficient and cost-effective pectin extraction methods.

- Green extraction technologies used on both wet and dry pomace surpassed the 15% pectin yield typically obtained by conventional acid extraction.
- Extraction methods influence pectin characteristics: enzymatic extraction favoured low-methoxyl pectin (used in jams and jellies), while physical methods favoured high-methoxyl pectin (used in low-sugar products and pharmaceuticals).
- Future tests will include using DES on wet pomace, which may provide optimal environmental and economic conditions.

References:

- **Advances in the pectin production process using novel extraction techniques: A review.** Food Hydrocolloids, 62, 239-250. Lanrewaju Ridwan Adetunji, Ademola Adekunle, Valérie Orsat & Vijaya Raghavan (2017).
- **Reconsidering conventional and innovative methods for pectin extraction from fruit and vegetable waste: Targeting rhamnogalacturonan I**. Trends in Food Science & Technology, 94, 65-78. Guizhu Mao, Dongmei Wu, Chaoyang Wei, Wenyang Tao, Xingqian Ye, Robert J. Linhardt & Shiguo Chen (2019).
- **Radio frequency assisted extraction of pectin from apple pomace: Process optimization and comparison with microwave and conventional methods.** Food Hydrocolloids, 121. Zheng Jianjun,Li Hongyue, Wang Dongchi, Li Rui,Wang Shaojin & Ling Bo (2021).
- **Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents**. Comptes rendus - Chimie,21(6),628-638. Henni Vanda, Yuntao Dai, Erica G. Wilson, Robert Verpoorte & Young Hae Choi (2018).

2.3 Best practices in the production of tomato pomace, peels and seed for valorization purposes

Contributor: TomaPaint

Around 150,000 tonnes of tomato processing waste are produced each year in Italy alone. Tomato waste, mainly composed of peels and seeds has the potential to be used as a feedstock for biobased products. However, its high moisture level poses challenges for transportation and reduces stability over time. Since peels and seeds offer distinct valorization opportunities, TomaPaint has developed a process to separate them efficiently and reduce the water content, thereby enhancing the value of the extracts and improving their resistance to microbiological degradation.

Project activity: As part of the AgriLoop project, TomaPaint has successfully developed a sustainable, two-stage process to dehumidify tomato processing waste. First, peels and seeds are separated efficiently using a flotation system that leaves a residual seeds concentration in the resulting product of less than 10%. This equipment is 30m long and can treat more than one ton of by-products per hour.

The second stage of the TomaPaint process uses excess heat coming from an associated biogas plant (a sustainable and renewable energy source), to dry the peels and seeds up to a residual humidity of less than 6%.

Peels and seeds are separated to enable distinct valorisation: peels for example contain cutin (a polymer used in applications such as paints), while seeds are used for oil production. Separating them before processing improves extraction yields and purity compared to processing mixed pomace.

	Pomace		Floated Peels		Floated Seeds	
	Wet	Dried	Wet	Dried	Wet	Dried
Humidity % (w/w)	71.7	3.3	76.4	4.2	72.1	5.2

Key findings and recommendations: The Tomapaint process has the following benefits:

- Efficient separation of tomato peels and seeds.
- Microbiological stabilization of the product over time.

- Low carbon dioxide emissions due to the use of excess heat from a biogas plant for drying.
- Reduced transportation costs.

2.4. Production efficiency and functional characteristics of dietary fiber and peptides from potato processing waste

Contributor: Mengmei Ma (IFST-CAAS)

Potato is the fourth most important food crop worldwide. In China, potatoes are mainly used for starch production, which generates large amounts of waste. For every ton of starch produced, 4.5-5.0 tons of wet peel residues and 10-20 tons of wastewater are generated. These by-products contain valuable dietary fiber and nutrients, yet only a small portion is used as animal feed and the rest is discarded, resulting in wasted resources and environmental pollution. The AgriLoop project is developing green extraction technologies to recover high-value food components from potato processing waste.

Project activity: The effects of different pretreatments including ultrasound, microwaves, and ultrafine grinding on the extraction efficiency and composition of active compounds were investigated. Advanced methods such as Natural Deep Eutectic Solvents (NADES), high-hydrostatic pressure, enzymatic hydrolysis, and ultrasound/microwave-assisted extraction were assessed to identify optimal strategies for extracting dietary fiber, peptides, and polysaccharides.

Analytical techniques, including ion chromatography, Fourier Transform Infrared Spectroscopy (FTIR), and nuclear magnetic resonance (NMR) were used to identify the correlation between chemical composition, physicochemical properties, structural characteristics, and biological function (e.g. lipid-lowering, sugar regulation, modulation of gut microbiota).

Key findings and recommendations:

Potato residues are a rich source of dietary fiber and peptides and could be valorized through enzymatic and physical modification to produce high-value functional ingredients.

- >90% purity potato dietary fiber can be obtained via enzymatic hydrolysis.

- High-hydrostatic pressure (400 MPa) combined with cellulase (270 U/g) increased soluble dietary fiber yield by 59.3%.
- Modified potato dietary fiber showed strong adsorption of cholesterol, glucose, and amylase.
- The optimal protease combination for peptide production was ALC (4%) + NEU (4%) + FLA (4%), with EGU-40 ultrasound as the most effective treatment.
- Potato peptides exhibited high antioxidant activity (82.24 μg TE/mL) with a recovery rate of 69.09%.

References:

- **Nutritional composition, antioxidant activity, volatile compounds, and stability properties of sweet potato residues fermented with selected lactic acid bacteria and bifidobacteria.** Food Chemistry, 2022, 374, 131500.
- **Effect of high hydrostatic pressure-assisted pectinase modification on the Pb^{2+} adsorption capacity of pectin isolated from sweet potato residue**. Chemosphere, 2021, 262, 128102.

2.5 Turning agricultural anaerobic digesters into multiproduct biorefineries

Contributor: David Bolzonella

More than 18,000 anaerobic digestion (AD) plants are currently operating in the EU. As the incentive schemes for power generation come to an end in several European countries, the exploitation of anaerobic digesters needs to be reconsidered. Eighty per cent of digesters are rural; turning crop residues and manures into energy and fertilisers. These agricultural digesters have the potential to be reconfigured into sustainable multi-feedstock and multi-purpose biorefineries that can transform agro-waste into valuable bio-based products.

Project activity: The AgriLoop project has designed and tested a complete, AD-based biorefinery at pilot scale where agro-wastes are transformed into bioproducts in a real farm environment.

The platform is comprised of the following main areas:

- agro-waste pre-treatment and preparation

- acidogenic fermentation
- mixed microbial biomass selection and natural polyesters (PHA) storage
- PHA extraction and purification
- anaerobic digestion of the solid part of the fermentation effluent
- recovery of nutrients
- microbial protein production

When 1m^3 of slurry at 20% dry matter was treated every day, up to 2 m^3 of hydrogen and 18 m^3 of methane could be produced. Forty kg of volatile fatty acids (VFA) and up to 8 kg of PHA could also be extracted, as well as 0.6 kg and 0.05 kg of N and P.

Key findings and recommendations:

There is an urgent need for reconfiguring agricultural anaerobic digesters into biorefineries that convert agro-waste into valuable bio-based products.

This study has demonstrated a pilot-scale biorefinery using agri food wastes to produce mainly; biofuels (methane and hydrogen), volatile fatty acids (VFAs), PHAs and microbial mass proteins. However, the process is highly flexible, allowing the operator to opt for different bioproduct outputs.

The practical set up of this platform will be further studied and its applicability analysed. Environmental, social and economic benefits will also be assessed.

References:

- **Toward the Transition of Agricultural Anaerobic Digesters into Multiproduct Biorefineries.** Authors: David Bolzonella, Davide Bertasini, Riccardo Lo Coco, Miriam Menini, Fabio Rizzioli, Anna Zuliani, Federico Battista, Nicola Frison, Aleksandra Jelic and Giovanna Pesante. https://www.mdpi.com/2227-9717/11/2/415

2.6 A novel, low-cost, continuous process for producing PHA using agricultural wastes as a substrate for mixed microbial cultures

Contributors: Mariana Villano (Sapienza University of Rome) Lionel Nguemna Tayou (Sapienza University of Rome), Flavia Marzulli, (Sapienza University of Rome), Gaia Salvatori (Sapienza University of Rome), Claudia Vona (Sapienza University of Rome)

Bioplastics offer a promising alternative to the pollution problems caused by conventional plastics. Polyhydroxyalkanoates (PHAs) are a fully biodegradable family of bioplastics, that can be produced by bacteria. They are suitable for many applications but are more expensive to produce. The AgriLoop project is investigating a novel, low-cost, continuous process for producing PHA using agricultural wastes as a substrate for mixed microbial cultures (MMC).

Project activity: A laboratory-scale, continuous, MMC-PHA process has been developed, consisting of three stages: aerobic microbial selection, PHA accumulation and anaerobic quenching.

Microbial selection is via a continuously fed feast reactor linked to a continuously stirred famine reactor. From there, part of the biomass is recirculated to the feast reactor, and the rest goes to a continuously fed PHA accumulation reactor. The PHA-rich biomass is continuously sent to a quenching tank that inhibits microbial activity via acidic or thermal shock. Over 170 grams of the Poly(hydroxybutyrate-hydroxyvalerate) (PHBV) copolymer was recovered, with purity from 65 to 92%, wt/wt and interesting characteristics for future applications. These initial tests used synthetic mixtures of carboxylic acids as feedstock, but future activities will test the use of agri-food wastes (e.g. fermented wine lees and potato peels).

Key findings and recommendations: The research found that a continuous MMC-PHA production process is recommended over conventional batch processes to achieve the following benefits:

- The feast and famine phases of microbial selection are spatially separated, avoiding the need for computerized monitoring to control the reactor phases or an oxygen probe to identify the end of the feast phase.
- The ratio between the recirculation flow rate between the feast and famine reactors and the influent flow rate to the feast rector can be adjusted to maximize PHA productivity.

- High PHA purity and high molecular weight can be obtained with a chlorine-free extraction procedure.

References:

- **Chlorine-free Extractions of Mixed-Culture Polyhydroxyalkanoates Produced from Fermented Sewage Sludge at Pilot Scale.** Industrial and Engineering Chemistry Research, 62(43). Salvatori, G., Alfano, S., Martinelli, A., Gottardo, M., Villano, M., Ferreira, B. S., Valentino, F., & Lorini, L. (2023).https://doi.org/10.1021/acs.iecr.3c02684
- **Recirculation factor as a key parameter in continuous-flow biomass selection for polyhydroxyalkanoates production.** Chemical Engineering Journal, 455. Tayou Nguemna, L., Marzulli, F., Scopetti, F., Lorini, L., Lauri, R., Pietrangeli, B., Crognale, S., Rossetti, S., Majone, M., & Villano, M. (2023). https://doi.org/10.1016/j.cej.2022.140208

2.7. Valorization of grey starch from the potato processing industry

Contributor: Korneel Rabaey (UGent)

The potato processing industry is growing rapidly in the EU. During heat processes such as blanching, side streams are created that are high in starch but unsuitable or unattractive for use in feed or food. Over one million tons of this 'grey starch' are produced in the EU every year. Grey starch has potential as a carbohydrate source for novel feed products such as microbial protein. The AgriLoop project is developing an effective microbial protein production process based on grey starch.

Project activity: Grey starch from potato processing is first hydrolyzed to release the sugars and then fermented to produce organic acids. All types of organics present in the grey starch can be used by a microbiome to create a mixture of organic acids.

After filtration, this mixture can be used as a substrate for a single type of microorganism. Alternatively, the sugar-rich substrate can be used directly by a single microorganism. This is a simpler process but does not use all the organics from the grey starch. We intend to dry the microbial protein produced and provide to a feed company who will test its performance as fish food.

Key findings and recommendations: From these tests, we conclude that:

- Direct microbiome-based fermentation of grey starch is possible but difficult due to the substrate's high viscosity. Dilution is needed, which causes lower substrate concentrations downstream.
- Grey starch can effectively be hydrolyzed with enzymes to produce a concentrated sugar solution.
- The sugar solutions obtained can be used by a single microorganism at laboratory scale, and we are now identifying key growth parameters for scale up.
- Mixtures of organic acids, created via the fermentation of the grey starch, can be used by a single microorganism to produce microbial protein. The quality of the product depends on the types of organic acid.

References:

- **Decoupling Livestock from Land Use through Industrial Feed Production Pathways.** Environ Sci Technol 52:7351–7359. Pikaar I, Matassa S, Bodirsky BL, Weindl I, Humpenöder F, Rabaey K, Boon N, Bruschi M, Yuan Z, van Zanten H, Herrero M, Verstraete W, Popp A (2018) https://doi.org/10.1021/acs.est.8b00216
- **Impact of substrate and growth conditions on microbial protein production and composition.** Bioresour Technol 317:124021. Sakarika M, Candry P, Depoortere M, Ganigué R, Rabaey K (2020) https://doi.org/10.1016/j.biortech.2020.124021

2.8. Developing agro-residue biocomposites to meet the needs of farmers

Contributor: Tatiana Coutand

Plastics are omnipresent in agriculture for a broad range of applications, but their use and degradation results in persistent micro- and nano-plastic particles that contaminate soils and affect crop production. To tackle this issue, we are developing bio-based and biodegradable composite materials made from agro-residues. Our frugal design approach involves end-users, mainly farmers, from the earliest stage to ensure the materials possess only the necessary functional properties, avoiding the unnecessary replication of conventional plastics. However, agro-residues may contain residual contaminants, such as phytosanitary substances, which can persist through processing and pose risks if returned to the soil. To address this, we introduce a crucial new perspective by integrating safety criteria into the trade-off analysis between material performance, cost and environmental impact.

Project activity: Biocomposites using PHBV* as the matrix and finely ground peanut shell** particles (up to 15% by weight) as fillers, have been developed at the University of Montpellier. Rigid items were then manufactured by injection moulding.

To evaluate the safety of these materials, a 'challenge test' methodology was implemented. This involved deliberately contaminating the raw peanut shells with a range of pesticides, then assessing their capabilities throughout the transformation processes, including successive grinding steps and the subsequent production of the composite materials.

*PHBV: Polyhydroxybutyrate-co-hydroxyvalerate, a bio-based and biodegradable polymer (plastic) produced by microorganisms. **Raw peanut shells were supplied by Shandong Jinsheng Cereals & Oils Foods Co. Ltd, partner of the project.

Key findings and recommendations: This technology has delivered encouraging results and demonstrates potential as a cost-effective solution for transforming agricultural residues, especially blends of PHBV with peanut shells, into valuable bio-based materials. Replacing conventional plastics with bio-based materials could solve many problems.

- When these materials biodegrade, they don't produce any persistent particles because the carbon returns to the soil, meaning there's no plastic contamination of the land.
- Using agro-residues avoids the problems of managing this resource as waste. But the residual contamination of the new products obtained from these potentially contaminated residues must be carefully considered, as they could create new environmental issues.
- Involving the end-users, such as farmers, in the early design stage can help to create end-products tailored to specific requirements rather than trying to imitate conventional ones. In addition, including stakeholders helps to raise their awareness of current issues and possible solutions.

2.9. Economic and life cycle evaluation of the peanut supply chain in North China

Contributor: Gang Li (BTBU)

The agriculture and food industry often has long, complex supply chains that hide significant, underreported carbon emissions. Peanut oil production exemplifies this complexity, involving cultivation, transportation, processing, and distribution before reaching consumers. A systematic quantitative assessment remains lacking. This study aimed to provide the peanut oil industry with a clear, data-driven "roadmap" for green development of the whole peanut industry chain by:

- Quantifying the carbon emissions and composition of peanuts from planting to sales
- Identifying key emission links and analysing the main influencing factors
- Recommending targeted emission reduction paths.

Project activity: We applied a Multi-Region Input-Output (MRIO) model that traces how the purchase of a bottle of peanut oil in a Beijing supermarket drives upstream production activities, while calculating associated carbon emissions at each stage.

Our model breaks the supply chain down into agriculture, processing, transportation, warehousing, and wholesale/retail sectors. This framework generates a comprehensive database linking inter-industry transactions, final demand, and carbon emission intensities across 87 cities.

It found that carbon emissions in the peanut oil supply chain are marked by high concentration and significant spatial separation. Processing is the core emission driver in the supply chain. Production-based carbon emissions (PBAs) are highly concentrated in cities such as Junan and Nanyang. In contrast, consumer-based carbon emissions (CBAs) are broadly distributed across urban centres like Beijing and Tianjin, where emissions are low, but consumption is high. These cities are net carbon importers, appearing "clean" locally while shifting significant emissions responsibility to distant production regions.

Key findings and recommendations: Our preliminary policy recommendations are:

- At the production end: promote energy-efficient technologies in processing centers and encourage green agricultural practices

- At the consumption end: promote green consumer behaviour and develop cross-regional ecological compensation mechanisms to equitably redistribute environmental responsibility.

References:

- **Climate change and food systems.** Annual Review of Environment and Resources, 37, 195–222. Vermeulen, S. J., Campbell, B. M., & Ingram, J. S. I. (2012).
- **Life cycle assessment of soybean and palm oil-based biodiesel production in Brazil: A review.** Journal of Cleaner Production, 269, 122336. Talita, A., Silva, G., & Ometto, A. R. (2020).
- **Systematic review of greenhouse gas emissions for different fresh food categories.** Journal of Cleaner Production, 140, 766–783. Clune, S., Crossin, E., & Verghese, K. (2017).
- **Environmental and social footprints of international trade.** Nature Geoscience, 11(5), 314–321. Wiedmann, T., & Lenzen, M. (2018).

2.10. Metabolic engineering of *Pseudomonas putida* for efficient production of PHA from lignin derivatives

Contributor: Chao Yang (Key Laboratory of Molecular Microbiology and Technology for Ministry of Education)

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that offer a sustainable alternative to conventional petrochemical-based plastics. However, their widespread adoption is hindered by high production costs. *Pseudomonas putida* (strain KT2440), a soil bacterium with natural aromatic catabolic pathways, is a promising microbial chassis for converting lignin-derived compounds into medium-chain-length PHA (mcl-PHA). This study aimed to enhance this bioconversion process and reduce production costs through genetic engineering and bioprocess optimization.

Project activity: A genome-reduced derivative, *P. putida* (strain KTU-U27) — previously shown to exhibit superior PHA productivity compared to its parental KT2440 strain —was further engineered to improve mcl-PHA synthesis from the lignin-derived aromatic compound p-coumaric acid (p-CA). The following genetic modifications were introduced:

- Inhibition of PHA degradation by deleting the phaZ gene, which encodes the enzyme responsible for intracellular PHA breakdown.

- Suppression of fatty acid β-oxidation through deletion of fadBA1 and fadBA2 genes.
- Enhanced PHA biosynthesis via overexpression of phaC1 and alkK, key genes in the PHA production pathway.
- Enhanced resistance to substrate toxicity by overexpressing the ttg2ABCDE efflux operon and the vacJ genes.

Bioprocess optimization of the carbon-to-nitrogen (C/N) ratio and high-density fed-batch fermentation further improved PHA productivity.

Under optimized conditions (initial C/N ratio of 8:4), the final engineered strain (KTU-U27△Z2BA-P46C1K-P46TJ) achieved:

- Cell dry weight: 2050 mg/L
- PHA content: 82.19 wt%
- PHA yield: 1685 mg/L

Key findings and recommendations: This study demonstrates that an integrated strategy—combining genome reduction, metabolic pathway engineering, and bioprocess refinement—can substantially improve the bioconversion efficiency of lignin-derived aromatics to mcl-PHA.

The engineered strain significantly improved the performance of *P. putida* KTU-U27, achieving the highest reported yield of mcl-PHA using p-CA as the sole carbon source.

The enhanced *P. putida* KTU-U27 platform provides a scalable, cost-effective solution for sustainable PHA production from lignin, improving the viability of bio-based plastics.

References:

- **Systems metabolic engineering of genome-reduced *Pseudomonas putida* for efficient production of polyhydroxyalkanoate from p-coumaric acid.** J. Agric. Food Chem. 73(21):12899–12907.Chen Y, Liu Y, Meng Y, Jiang Y, Zhang X, Liu H, Reis MAM, Qi Q, Yang C, Liu R, 2025.

2.11. Digital carbon emission accounting and economic evaluation for effective agricultural waste management

Contributor: XiaoLong Yao (BTBU)

Effective agricultural waste management requires precise carbon emission accounting and economic evaluation to support informed decision-making by farmers, agricultural cooperatives, biorefinery operators, and environmental policymakers. We have developed an intelligent, automated system, capable of handling the intricate calculations required for assessing agricultural waste-to-value conversion projects.

Project activity: The approach taken involved:

- Developing Python-based, analytical software with a graphical, user-friendly interface (GUI).
- Integrating 13 distinct carbon emission calculation models (covering: fossil fuel combustion nitrogen oxide emissions, manure fermentation, wastewater treatment purchased electricity/heat, industrial/municipal wastewater, solid waste management, and distiller's grain fermentation).
- Creating economic evaluation modules for cost analysis, revenue calculation, profit analysis, and return on investment (ROI).
- Implementing sensitivity analysis functionality to assess how key factors affect economic viability.
- Designing automated reporting features (charts, graphs and exportable data tables).
- Enabling Excel data integration.

The software reduces the calculation time for carbon accounting of complex agricultural waste projects from days to minutes and improves accuracy by around 95%.

Sensitivity analysis reveals product pricing and raw material costs to be the most critical factors affecting project viability, with SAF (Sensitivity Analysis Factor) values typically ranging from 0.8-1.2.

Integrated economic-environmental analysis reveals hidden opportunities where waste conversion can be optimised.

Automated data visualization makes complex data accessible to non-technical stakeholders and improves their understanding and acceptance of complex technical data.

Key findings and recommendations: The software developed highlights the transformative role of digital tools in carbon accounting and economic evaluation for agricultural waste management.

- Faster and more accurate at carbon accounting
- More accessible to non-technical stakeholders
- Enables quicker, better decision-making through real-time scenarios, sensitivity analysis and integrated economic-environmental analysis.
- Widely applicable, with modular software design making it easily adaptable to different agricultural waste streams, regional specificities and scales.

References:

- National Key Program of China: International Science and Technology Innovation Cooperation Project (2023YFE0104900)
- AgriLoop Project Deliverables on agricultural waste valorization
- IPCC Guidelines for National Greenhouse Gas Inventories (2006)
- European Union Best Available Techniques Reference Documents
- Relevant publications in Bioresource Technology, Journal of Cleaner Production, and Chemical Engineering Journal

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2.12 Bioplastics production with the bacterium *Halomonas cupida J9*

Contributor: Chao Yang (Key Laboratory of Molecular Microbiology and Technology for Ministry of Education)

Microbial production of polyhydroxyalkanoates (PHAs) offers significant environmental benefits as a biodegradable alternative to petroleum-based plastics. However, commercial viability remains limited by high production costs, primarily due to the reliance on expensive carbon sources and the need for high-temperature sterilization during the fermentation of pure cultures. This study aimed to develop a low-cost PHA production platform using *Halomonas cupida*, a halophilic bacterium that naturally synthesizes PHA and thrives under high-salinity, avoiding the need for sterile conditions.

Project activity: A markerless genome-editing system was developed for *H. cupida* strain J9. The strain was genetically engineered to enhance xylose utilization for PHA biosynthesis through the introduction of a synthetic xylose metabolism module and the elimination of competing xylonate production by gene deletion.

The engineered strain, J9UΔxylD-P8xylA, achieved a PHA yield of 2.81 g/L using xylose as the sole carbon source—the highest yield reported to date for a *Halomonas* strain under these conditions. Notably, the strain produced a mixed PHA containing both short-chain-length (SCL) and medium-chain-length (MCL) monomers.

Other results include:

- The engineered strain was capable of efficient co-utilization of glucose and xylose for PHA production.
- In fed-batch fermentation with a glucose/xylose co-feeding strategy, PHA production reached 12.57 g/L in a 5-L bioreactor under open, non-sterile conditions.
- When cultivated on corn straw hydrolysate (a lignocellulosic agricultural waste product), the engineered strain reached a cell dry weight (CDW) of 7.0 g/L and produced 2.45 g/L PHA under open fermentation conditions.

Key findings and recommendations: The engineered strain J9UΔxylD-P8xylA achieved the highest reported PHA yield (2.81 g/L) by *Halomonas* using xylose as the sole carbon source.

This is the first report of *Halomonas* synthesizing SCL-co-MCL PHA (short- and medium/long-chain-length monomers) from xylose. These copolymers have valuable thermoplastic and elastomeric properties, making them valuable for a wide range of applications.

The system developed here demonstrates the feasibility of low-cost PHA production from lignocellulose-rich agricultural waste, enabled by non-sterile production conditions and the use of inexpensive feedstocks.

References:

- **Establishment of low-cost production platforms of polyhydroxyalkanoate bioplastics from *Halomonas cupida* J9.** Biotechnol. Bioeng. 121(7):2106–2120.Wang S, Liu Y, Guo H, Meng Y, Xiong W, Liu R, Yang C, 2024.

2.13. Technologies for anaerobic fermentation of organic waste

Contributor: Mingxiong He (BIOMA)

AgriLoop is developing more efficient and cost-effective technologies to transform agricultural residues, such as vinasse (a byproduct of the sugar and ethanol industries) and spent mushroom substrate, into valuable resources for sustainable, biobased production.

Project activity: This project developed and optimised a process where pretreatment of agricultural waste was followed by enzymatic hydrolysis and saccharification and the subsequent production of bioethanol, lactic acid, biogas, and biofertilizer.

The study included:

- Analysis of organic waste composition (total and volatile solids, C, N, C/N, pH, cellulose, hemicellulose, lignin and ash).
- Selection and screening of over 500 microbial strains for waste conversion into biogas and biofertilizer
- Composting of corn straw, biogas slurry, and spent mushroom substrates to produce biofertilizer.
- Assessment of organic fertilizers for crop yield, soil health and food safety.

Our study found that:

Pretreatment and enzymatic saccharification effectively broke down complex biomass structures, such as cellulose, hemicellulose and lignin, yielding 15.71–160 g/L of fermentable sugars.

Spent mushroom substrate combined with corn straw produced up to 53.35 g/L of ethanol in 5-L fermenters.

Cumulative methane yield from crop straw and animal manure -reached 120 mL/g volatile solids, a 6.5% improvement over the control.

Ethanol fermentation residues were repurposed for anaerobic digestion, improving process efficiency by producing up to 37.75 g/L ethanol and achieving a biogas yield of 337.38 mL/g total solids.

Selective breeding of *Bacillus coagulans* produced improved strains for lactic acid fermentation, achieving 1 ton of lactic acid from 3 tons of corn straw.

Composts produced from corn straw, biogas slurry, and spent mushroom substrates were found to significantly promote plant growth, crop yield and grain weight.

Key findings and recommendations

This study has developed promising technologies that should be considered as possible routes to more efficient, cost-effective conversion of agricultural residues into high-value products.

- Enzymatic hydrolysis and saccharification of cellulose and hemicellulose are critical for sugar release and the subsequent production of ethanol, lactic acid, and other bio-based products.
- High titre ethanol production from spent mushroom substrate combined with corn straw has been established.
- Biogas production from ethanol fermentation residues enhances overall process efficiency, with higher ethanol and biogas yields.
- Biofertilizer derived from composted residues improves soil organic matter and provides essential nutrients, contributing to sustainable agriculture.

References:

- Spent Mushroom Substrates Shifting of Bacterial and Fungal Dynamics During the Composting of Straw and Biogas Slurry, 2025, submitted.
- High Titer Ethanol Production from Spent Mushroom Substrate Combined with Corn Stover at High Solid Loading, 2025, submitted.
- The Multi-improving Effects of A Synthesized Microbiome upon corn straw silage and Microbial Community. 2025, under review.
- A high-temperature resistant cellulose degrading bacterium and its application. Patent, CN202411429444.7

Conclusion

The second batch of Best Practice Abstracts strengthens AgriLoop's commitment to translating complex research and innovation into clear, practical outcomes. By using a consistent framework—including a detailed guide, template, and established structure—the project has ensured that its technical findings are both accurate and readily digestible by a broad audience, from researchers to farmers and SMEs.

Taken together, these 13 abstracts demonstrate the practical feasibility of applying green chemistry, advanced research principles and software solutions to valorize agricultural byproducts. They confirm the potential for optimised, resource-efficient processes to yield commercially relevant bioactive compounds.

These insights are important for validating the circular bioeconomy models proposed by the project, providing confidence that the creation of new, economically viable, and sustainable value chains from residues is achievable.

Dissemination

To reach the scientific community, the abstracts will be published on the EU CAP Network portal and project website, while also being shared through partner institutional repositories, at relevant training events and through European research networks such as the Rural Bioeconomy Alliance. For practitioners and end users—including farmers, industry, and agricultural advisors—the abstracts will be distributed in an accessible A4 2-page format (see Annex 2) at relevant workshops, agricultural fairs, and during the final stakeholder event in October 2026.

Activity will be planned for in M40 to maximize uptake of results. This will see the dissemination strategy tailored to different stakeholder groups, with technical abstracts prioritized for research institutions, policy related abstracts targeted at policymakers and economically focused ones highlighted for industry and farming cooperatives. Beyond the project's conclusion, lasting accessibility will be ensured through permanent hosting on the EU CAP Network. This approach provides a strong foundation for concluding project results and developing final policy and industry recommendations in forthcoming deliverables.

Annex 1: Guidance materials and template

BEST PRACTICE ABSTRACT

GUIDELINES

Crafting an effective best practices document requires a clear and thoughtful approach, especially within the complex landscape of agriculture and biorefining. This guide will help you create a compelling and accessible document that resonates with diverse stakeholders.

According to our Grant Agreement, the Best Practice Abstracts can be positioned within these categories:

1. **Good farming practice documents** grouped by crop or livestock system and adapted to different agro-climatic regions to ensure broad representation across EU and CN.
2. **Best practice biorefinery recommendations**, covering the most promising by-product and co-product processing technologies and biorefinery options—organised by product groups (e.g. biomaterials, agricultural supplies, food additives).
3. **Policy recommendations**, with a focus on opportunities linked to the Green Deal, EU Bioeconomy Strategy, Farm-to-Fork Strategy, and the EU-China FAB initiative—formalised through policy briefs, direct interaction with policymakers, and participation in relevant events.
4. **Recommendations for implementing an EU-CN shared SSbD vision** for agri-residue management.

Key principles:

- Develop a focused, concise vision of your best practice
- Use clear, straightforward language
- Communicate technical concepts in accessible terms
- Aim for brevity and clarity (approximately 2 pages)
- Organize content with short, focused paragraphs
- Enhance understanding with supporting visuals and diagrams

1. CONTEXT

Providing context is crucial. Readers connect more deeply when they understand the background and significance of your work. Consider adding:

- Location and setting of the practice
- Type of farm, process, or crops involved
- Brief literature review and/or situational summary
- Specific challenges or opportunities addressed
- Target beneficiary groups (farmers, researchers, industry)
- Key stakeholders involved

Explain the original motivation: What process were you improving? What specific issues were you addressing? How might this practice transform current approaches?

2. PROJECT ACTIVITY

Provide a concise, practical description of what was implemented in the field, biorefinery or laboratory. Focus on delivering a clear narrative that captures the essence of your work:

- Explain the specific actions taken
- Highlight tangible results and impacts
- Demonstrate measurable outcomes

Key areas to address include: quantitative changes (e.g. did crop yields increase?); environmental impact (e.g. did soil health improve?); efficiency gains, etc. Here, it's important to provide specific, measurable data points where possible.
Visuals are also crucial to effective communication. Ensure you use high-quality images, clear graphics and diagrams, and illustrate key points with compelling visual evidence.

In this section, you can also go beyond a single context by exploring broader applications, considerations, and constraints, including:

- The potential for adaptation in different environments
- Specific requirements for successful replication
- An honest assessment of any limitations

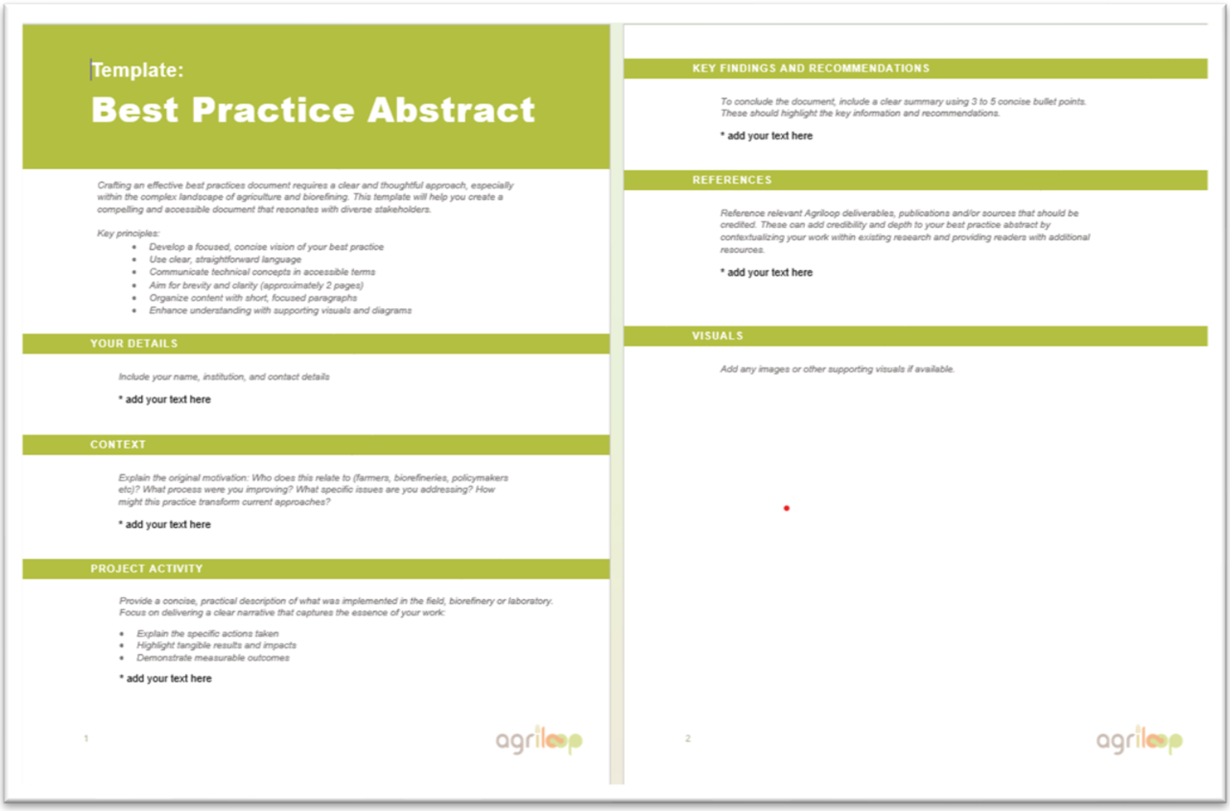
The goal is to create a comprehensive yet accessible description that demonstrates the practice's value, versatility, and potential for broader impact.

3. KEY FINDINGS

To conclude the document, include a clear summary using 3 to 4 concise bullet points. These should highlight the key information and recommendations.

4. REFERENCES

Reference relevant Agriloop deliverables, publications and/or sources that should be credited. These can add credibility and depth to your best practice abstract by contextualizing your work within existing research and providing readers with additional resources.



Template:

Best Practice Abstract

Crafting an effective best practices document requires a clear and thoughtful approach, especially within the complex landscape of agriculture and biorefining. This template will help you create a compelling and accessible document that resonates with diverse stakeholders.

Key principles:

- *Develop a focused, concise vision of your best practice*
- *Use clear, straightforward language*
- *Communicate technical concepts in accessible terms*
- *Aim for brevity and clarity (approximately 2 pages)*
- *Organize content with short, focused paragraphs*
- *Enhance understanding with supporting visuals and diagrams*

YOUR DETAILS

Include your name, institution, and contact details

*** add your text here**

CONTEXT

Explain the original motivation: Who does this relate to (farmers, biorefineries, policymakers etc)? What process were you improving? What specific issues are you addressing? How might this practice transform current approaches?

*** add your text here**

PROJECT ACTIVITY

Provide a concise, practical description of what was implemented in the field, biorefinery or laboratory. Focus on delivering a clear narrative that captures the essence of your work:

- *Explain the specific actions taken*
- *Highlight tangible results and impacts*
- *Demonstrate measurable outcomes*

*** add your text here**

1

KEY FINDINGS AND RECOMMENDATIONS

To conclude the document, include a clear summary using 3 to 5 concise bullet points. These should highlight the key information and recommendations.

*** add your text here**

REFERENCES

Reference relevant Agriloop deliverables, publications and/or sources that should be credited. These can add credibility and depth to your best practice abstract by contextualizing your work within existing research and providing readers with additional resources.

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VISUALS

Add any images or other supporting visuals if available.

2

Annex 2: Example A4 2 page Practice Abstract

Best Practice

November 2025

Extraction efficiency of polyphenols from peanut red skin

Peanut is a key oil crop in China and its seed coat (peanut red skin) is an important by-product of peanut processing. Each year, more than 400,000 tons of peanut red skin are generated in China, yet its valorisation remains limited. Notably, peanut red skin is rich in bioactive phenolic compounds, including proanthocyanidins and resveratrol, with high nutritional and functional value. Natural deep eutectic solvents (NADES) have emerged as promising, environmentally friendly solvents for extracting such compounds.

NADES are formed by mixing hydrogen bond donors and acceptors in defined molar ratios, offering easier preparation and biodegradability. Their dense hydrogen-bonding network efficiently extracts polyphenols while preserving structural integrity. They also provide enhanced safety and lower flammability versus conventional solvents like ethanol, reducing infrastructure costs. As such, using NADES for the extraction of polyphenols and other components from peanut red skin could enable high value uses for peanut processing waste.

Project activity

Ultrasonic-assisted extraction of polyphenols from peanut red skin was carried out using NADES in comparison with conventional solvents (methanol, ethanol, and water). Eight NADES formulations were prepared, with choline chloride as the hydrogen bond acceptor (HBA) and ethylene glycol, 1,2-propanediol, glycerol, lactic acid, citric acid, urea, glucose, and sucrose as the hydrogen bond donors (HBDs). Extraction efficiency was assessed by varying key parameters, including HBA/HBD molar ratio, solid-to-solvent ratio, water content in NADES, and ultrasonic conditions (time, temperature, power). Single-factor experiments were conducted by varying each parameter within the following ranges: extraction time (5–30 min), liquid-to-solid ratio (10–50 mL/g), choline chloride/lactic acid molar ratio (3:1 to 1:3), ultrasonic power (200–400 W), temperature (30–70 °C), and water content (10–50%). The process is currently being evaluated at pilot-scale.

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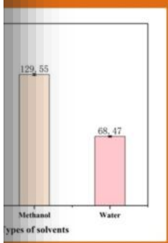

The optimal extraction conditions were identified as: choline chloride:lactic acid (1:1 molar ratio), 30% water content, liquid-to-solid ratio of 10 mL/g, ultrasonic power of 250 W, ultrasonic temperature of 40°C, and ultrasonic time of 20 min.

Under these conditions, the polyphenols yield reached 158.07 mg/g, representing a 2.3-fold increase over water extraction and 1.2-fold increase over alcohol extraction. In addition, polyphenols were recovered by static adsorption-desorption using macroporous resin, followed by freeze-drying and the resulting extracts exhibited strong in-vitro antioxidant activity.

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