單選題（共 50 題，每題 2 分，答錯倒扣 0.5 分，請作答於答案卡中）注意：請用 2B 鉛筆作答於答案卡

Article 1

(Source: *Gut Microbes*, 2024, 17(1):2442521.)

The human gut microbiota is a complex ecosystem composed of trillions of microorganisms that inhabit and interact with each other and the host throughout the gastrointestinal tract. This microbial community plays an important role in maintaining overall health and well-being by participating in a range of physiological processes, such as nutrient metabolism, immune modulation, and defense against pathogens. The presence and quantity of different beneficial microbes, each with specific functions, are key to maintaining a balanced ecosystem within the gut. In this sense, dietary fibers, as the main energy source for gut microbes, play a pivotal role in shaping the composition and function of the gut microbiota, ultimately influencing human health outcomes. Notably, microbes have different fiber degradation abilities and preferences, and even small differences in dietary fiber structures can lead to marked shifts in bacterial outcomes.

Dietary fiber refers mainly to a diverse group of plant-derived carbohydrates that resist digestion in the upper gastrointestinal tract and reach the large intestine intact. Although humans lack the enzymes necessary to break down fiber, it serves as a valuable energy source for gut microbes, which possess the enzymatic machinery to ferment most of these complex carbohydrates. Through fermentation, gut microbes convert fiber into various metabolites, including short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which are relevant due to their health-promoting effects locally and systemically. It is important to note that the structures of dietary fibers align with bacterial utilization capabilities. Dietary fibers differ in structural features, such as the degree of branching, glycosidic linkages, and solubility, which influence their fermentability by gut bacteria. While previous studies have explored the impact of individual dietary fiber types on gut microbiota modulation and SCFA production, few studies have focused on the collective effects of fiber mixtures. Evidence mapping of 141 publications revealed that most studies examined single fiber types, with only 7% investigating fiber combinations. We have previously reported that fiber mixtures may delay the fermentation rate in the large intestine, in agreement with previous clinical data showing that a mixture of fibers typical of a normal diet promotes more prolonged short chain fatty acid production compared to individual fiber sources. While some clinical benefits of fiber mixtures have been observed, no studies have investigated how mixtures can be made at a mechanistic level (i.e., how specific outcomes can be achieved through fiber mixtures). It, thus, remains unclear how human gut microbiota outcomes compare between mechanistically designed mixtures and their individual components. In synthetic communities, we have demonstrated that bacterial taxa employ various prioritization strategies for fiber utilization when present in a mixture, allowing them to coexist even in highly competitive environments, such as the gut microbiota. Moreover, given that individuals respond differently to different fiber types, it is likely that offering a broad range of fibers, as opposed to a single type of fiber, would be a more effective strategy to elicit consistent responses across different individuals. Still, most dietary interventions and investigations on gut microbial communities have focused on isolated fibers or specific fiber sources, overlooking the intricate interactions and possible synergistic effects that arise when multiple fiber types are consumed together. Thus, there is an important knowledge gap regarding how the gut microbiota responds to dietary fiber mixtures and the subsequent implications for SCFA production.

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Previously, we designed a fiber mixture that promoted different microbial groups and showed localized and systemic benefits to subjects with Parkinson's disease related to improvements in the gut community structure. Our hypothesis continues to be that systematically designed fiber mixtures provide a plethora of structures that can accommodate the metabolic requirements and preferences of a wide range of gut bacteria, thereby supporting a more balanced and diverse gut microbiota than any single fiber. We also hypothesized that fiber mixtures can render more consistent responses across people than single fibers. In this study, we aimed to elucidate the effects of systematically designed fiber mixtures compared to their single fiber components on human gut microbiota composition and SCFA production in vitro.

Please choose the best answer according to the above article:

1. What challenge does evidence mapping reveal about current dietary fiber research?
(A) lack of understanding of fiber metabolism (B) underrepresentation of systematic studies on fiber mixtures (C) high variability in microbial response to dietary fibers (D) limited knowledge on fiber fermentability.

2. What is a critical factor driving the hypothesis that fiber mixtures outperform single fibers for gut microbiota support? (A) enhanced nutritional value of mixtures (B) reduced pH fluctuations in the gut (C) broader structural diversity supporting different microbial taxa (D) simplified microbial interactions.

3. What is the rationale behind targeting health-related bacterial clusters in gut microbiota studies? (A) they directly consume dietary fats (B) they produce SCFAs beneficial for host health (C) they degrade proteins to enhance gut immunity (D) they outcompete pathogenic bacteria effectively.

4. How does the design of fiber mixtures align with the ecological principles of gut microbiota? (A) mimicking natural diversity in fiber availability (B) promoting homogeneity within microbial populations (C) encouraging competitive exclusion among microbial taxa (D) restricting microbial interactions to specific niches.

5. What critical feature of fiber mixtures enhances their ability to promote consistent microbial responses across individuals? (A) high solubility of all fiber components (B) elimination of gut microbial diversity (C) uniform fermentation rates across fiber types (D) synergistic effects of different fiber structures.

Article 2

(Source: *Nature Reviews Microbiology*, 2024, 22(9):528-542)

Microbial food spoilage represents organoleptic changes that render foods undesirable or unacceptable for consumption. Microbial food spoilage is typically caused by growth of microorganisms and the associated enzymatic activities; however, it can also be caused by microbially produced enzymes that are present in foods even after the corresponding microorganisms have been inactivated. Microbially mediated organoleptic changes of foods are wide ranging and may include off-odours, undesirable taste, physical and texture changes (for example, bloating and coagulation), as well as colour defects. Microbially spoiled food products

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often present with multiple defects, either because a given spoilage organism may cause multiple changes (for example, *Pseudomonas* spp. growth on a fresh cheese could cause both a blue colour and a bitter taste) or, less likely, because multiple spoilage organisms grew in a food (for example, due to improper temperature control). Although microbial spoilage of foods and microbial food safety issues are often conflated in the non-specialist literature and by the general public, microbial food spoilage and safety hazards are distinct, meaning food that carries microbial pathogens only very rarely shows concurrent signs of organoleptic spoilage and food that shows sign of spoilage will only rarely cause food-borne infection or intoxication (even though it may induce a 'gag reflex'). This review thus solely focuses on microbial food spoilage. Microbial food spoilage has substantial negative impacts on environmental sustainability and food security globally. Additionally, food spoilage can have major impacts on the food industry, such as reduced profitability or negative consumer reactions that may lead to market withdrawals. Importantly, social media allow individual consumers to easily share food spoilage events, which may require food companies to issue costly recalls or market withdrawals even if just a small proportion of product is impacted. Of note, defects that are easily captured visually (for example, with photographs or video) are particularly prone to 'viral' spread on social media. Examples include visual mould growth (for example, on yogurt) and changes in the consistency and shape of products (for example, package bloating due to growth of aerobic sporeformers, heterofermentative lactic acid bacteria (LAB) or yeast). Microbial food spoilage can be caused by a wide range of microorganisms, including bacteria and fungi. Although bacteriophages may also be considered a potential cause of microbial food loss or waste, this review focuses on bacterial and fungal causes of food spoilage. Common bacterial groups responsible for food spoilage include the genus Pseudomonas as well as other Gram-negative genera (for example, *Serratia*), aerobic and anaerobic sporeformers in the class Bacillales and the order Clostridiales, non-spore-forming Gram-positive thermoduric and LAB, as well as a wide range of yeast and moulds. These organisms show a range of metabolic activities (which, for example, can produce unwanted gas and acid) and can produce a range of enzymes (for example, proteases or lipases) that cause organoleptic defects. Spoilage microorganisms possess a wide range of adaptations that allow them to survive food processing treatments (for example, heat treatment) and grow in foods, such as an ability to grow under low pH and in high-salt and low-temperature conditions. The vast taxonomic and phenotypic diversity of microorganisms responsible for food spoilage represents a substantial challenge for detection, control and prevention of food spoilage. Hence, although the development of rapid tests has substantially improved our ability to detect and control food-borne pathogens (of which there are perhaps fewer than ten pathogens of major concern), this approach is unlikely to be successful against the diversity of food spoilage microorganisms.

The development of improved detection methods for spoilage microorganisms is further complicated as the relevant test targets may vary based on commodity, ranging from large groups (for example, psychrotolerant sporeformers across a range of genera) to very specific targets (for example, *Pseudomonas* spp. that produce blue pigments and specific heat-resistant moulds). The tremendous diversity of spoilage microorganisms also represents a challenge for prevention and control, particularly for targeted strategies (for example, specific antimicrobials and competitive cultures) as they often fail to target the necessary range of spoilage organisms. Hence, systems approaches that address contamination pathways from primary production through processing and distribution are needed to effectively reduce microbial food spoilage (and hence food waste). New systems modelling tools have the potential to allow for more rational design of systems that can

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effectively reduce food spoilage, while also minimizing unintended negative consequences.

Please choose the best answer according to the above article:

6. Why is it challenging to distinguish microbial spoilage from microbial contamination in public perception? (A) pathogenic and spoilage microbes may coexist but cause differing effects on food quality (B) spoilage and contamination both involve changes in pH and nutrient composition (C) the metabolic pathways of spoilage and pathogenic microbes are identical (D) both spoilage and pathogenic microbes induce visible structural changes in food.

7. Which microbial species is particularly notorious for causing both enzymatic and metabolic defects in dairy products? (A) *Bacillus cereus*, due to its thermoduric nature (B) *Listeria monocytogenes*, as it survives under refrigeration (C) *Clostridium perfringens*, by gas-forming metabolism (D) *Pseudomonas* spp., through production of lipases and proteases.

8. How has social media impacted the economic repercussions of microbial food spoilage? (A) it has accelerated public awareness of microbial contamination in food industries (B) viral spread of visually apparent defects, such as bloating and mold growth, forces companies to issue recalls even if spoilage is minimal (C) it has reduced the trust in global food safety practices, irrespective of actual spoilage events (D) social media has improved consumer education, lowering the frequency of food spoilage complaints.

9. Why is detecting microbial spoilage more complex than detecting foodborne pathogens? (A) spoilage microorganisms exhibit a wider range of physiological and metabolic adaptations (B) spoilage microorganisms rarely produce toxins, complicating their detection (C) detection methods for spoilage microorganisms are limited to enzymatic assays (D) foodborne pathogens are less diverse and easier to target with rapid detection kits.

10. Which approach is most likely to effectively reduce microbial spoilage across diverse food products? (A) implementation of commodity-specific rapid microbial detection technologies (B) use of generalized antimicrobial agents targeting both spoilage and pathogenic organisms (C) adoption of systems-based contamination pathway modeling from production to distribution (D) development of advanced enzymatic inhibitors targeting microbial metabolism.

Article 3

(Source: *Cell Reports*, 2023 42(7):112728)

For many years, mitochondrial functions were regarded as being restricted to the parental cell. However, several recent studies have pointed out that these organelles and their components can be released outside the cell into the extracellular milieu, under both physiological and pathological conditions.[16,17] Those components are secreted in a variety of forms and structures, including intact free, fragmented, and vesicle encapsulated, as well as free component like circulating mtDNA (cir-mtDNA), known as circulating cell-free mtDNA or ccf-mtDNA.[18] In a manner that highlights their novel signaling properties, these structures may interact with different types of cells. Because of their structural similarity to their bacterial ancestor, extracellular mitochondria and their components may operate as a danger signal by means of their interaction with pattern recognition receptors

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(PRRs) and thus stimulate undesirable inflammatory signaling pathways.[19,20] For instance, mtDNA, mitochondrial transcription factor A (TFAM), extracellular ATP, and numerous others have the capacity to elicit strong immune responses and, as such, are thus considered mitochondrial damage-associated molecular patterns (DAMPs).[21-24] Recent studies also report that, when discharged outside the cell, whether intact or damaged, whole mitochondria show considerable pro- or anti-inflammatory effects in different models, thus highlighting the paradoxical interactions between these organelles and immune cells.[25-28] It has also been shown, both *in vitro* and *in vivo*, that mitochondria and their components (short molecules, peptides, lipids, proteins, and nucleic acids) can be horizontally transferred between mammalian cells, resulting in changes to mitochondrial genes, bioenergetics profiles, and other functional characteristics of recipient cells.[29] This phenomenon was first described in a key study by Rustom *et al.*, who found that the delivery of these organelles occurs via a nanotubular network.[30] Extensive research subsequently revealed that, through this transfer, damaged cells can be rescued by the incorporation of exogenous mitochondria into their mitochondrial network. In the context of tumorigenesis, this horizontal transfer can also affect the functional capabilities of cancer cells, including their proliferation or resistance to chemotherapy. Thus, both positive and negative outcomes in recipient cells can result from this transfer.[31-34] In light of these studies, the direct transplantation of whole mitochondria into damaged areas has been suggested as a means of investigating the potential curative benefits.[35] The fundamental purpose of this review is to provide knowledge on the different forms of extracellular mitochondria and their by-products, on their interaction with recipient cells, and on that interactions' beneficial and detrimental effects.

Please choose the best answer according to the above article:

11. In this article, the author mentioned that mitochondrial function was regarded to be restricted to the parental cell. What do you think "parental cell" mean? (A) the nearby cells that provide nutrients for mitochondrial (B) the cells accompany mitochondria to exert their function (C) the cell in which mitochondria reside (D) all of the above.

12. In which of the following forms can mitochondria be secreted outside the cells? (I) intact form (II) partial mitochondria (III) vesicle encapsuled (IV) free mitochondria DNA. (A) I, II (B) I, III (C) II, IV (D) I, II, III , IV.

13. What might be the reason that released mitochondria can trigger inflammatory signaling? (A) they provide mtDNA (B) they provide protection (C) they provide chromosomes (D) they provide PRRs.

14. Mitochondria components that cause immune responses could be considered as (A) TFAM (B) DAMPs (C) PRRs (D) all of the above.

15. The discharged mitochondria can trigger which of the following effect? (A) pro-inflammatory effect (B) anti-inflammatory effect (C) both pro-inflammatory and anti-inflammatory effects are possible (D) only damaged mitochondria not intact mitochondria, would cause pathological effects.

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16. The components of mitochondria were described as having "paradoxical" interactions with immune cells. The word "paradoxical" can be replaced by which of the following words? (A) contradictory (B) convoluted (C) inscrutable (D) ambiguous.

17. In this article, what do the superscript numbers refer to? (A) the paragraph numbers (B) the reference numbers (C) the line numbers (D) the page numbers.

18. According the author's description, which of the followings is NOT a consequence of the horizontal transfer of mitochondria components between cells? (A) change in mitochondrial genes (B) change in proliferation status (C) change in drug resistant capacity (D) cause gene mutation.

19. What was Rustom's finding? (A) secreted mitochondria would cause immune responses (B) a novel structure called nanotubular network (C) mitochondria component would change after being transferred to another cell (D) cancer cells would be recognized by immune system after absorbing the foreign mitochondria.

20. What of the following do you think would NOT be included in this review article? (A) different forms of extracelluar mitochondria (B) the interaction between discharged mitochondria and recipient cells (C) the consequences of interaction between mitochondria and recipient cells (D) the way to prevent the secretion of mitochondria by-product.

Article 4

(Source: *Food Chemistry*, 2024, 452, 139570)

Resistant starch

Diabetes is a major chronic disease. Resistant starch (RS) refers to the starch that is hard to be digested or absorbed in the human small intestine. The increase in RS content in starch-rich foodstuffs has been proposed as a tool in the management of obesity and type 2 diabetes, since it escapes enzymatic digestion, and as a result, the postprandial glycemia is not altered. Currently, RS is categorized into five types: RS-1 (physically inaccessible starch), RS-2 (resistant granules), RS-3 (retrograded starch), RS-4 (physically or chemically modified starch) and RS-5. In the last almost 20 years, RS-5 is considered to be a resistant starch formed by complexation with lipids. In recent years, researchers have redefined RS-5. It is defined as the self-assembled starch V-type complexes, such as starch-glycerol, starch-amino acids, starch-peptides, starch-proteins, starch-lipid-protein, starch-polyphenols, starch-other polysaccharides.

α-amylase

In starch digestion, starch molecules are hydrolyzed by amylase into glucose and maltose, which are then absorbed by the body. The tertiary structure of α-amylase is briefly characterized to better understand the details of the complexation of starch molecules with α-amylase. Human α-amylase is a monomer protein consisting of 8 α-helices and 14 β-folds, with a radius of gyration of 2.33 nm, specific surface area of 193.7 nm^2, molecular weight of 55 kDa, and pI of 6.87. The active center is a catalytic triad consisting of ASP197, GLU233, and ASP300. Glu233 is responsible for releasing protons and attacking glycosidic oxygen, while ASP197 is responsible for the formation of the carbon ion intermediate and its stabilization. ASP300 is responsible for stabilizing the nucleophilic reagent (water molecule) and helping Glu233 extract protons from the water molecule to produce

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activated hydroxyl ions, which nucleophilically attack the carbon ion intermediate to complete the reaction. The surface of α-amylase has a cleft that is approximately 3.5 nm long and 1.5 nm wide, which is responsible for the binding of starch chains. In the middle of the active cleft is an active groove with a depth of about 1.4 nm. The catalytic triad is located at the bottom of the active groove. Root mean square fluctuation (RMSF) analysis results have shown that there are six highly flexible loop structures around the α-amylase active cleft. These highly flexible loop structures are closely related to substrate recognition and binding.

Starch-lipid interactions

Interactions between lipids and carbohydrates, which are major components of food, have been extensively studied. When starch is in a granular state, it can adsorb lipid molecules from the outside. The percentage of lipid molecules adsorbed by starch granules highly depends on their water content and temperature (granule swelling). As the water content and temperature increase, the lipid adsorption rate increases. When the starch is pasted and the granules disintegrate, the interactions between starch molecules and triglyceride molecules were analyzed by using molecular simulations. When a lipid molecule approaches a starch molecule, it wraps around the triglyceride molecule and forms a stable complex. Notably, starch molecules do not necessarily entangle the fatty acid chains completely (three turns); the vast majority of starch molecules entangled the fatty acid chains in only one turn (8 glucose residues per turn). Calculations revealed that the secondary interaction energy of glucose residues with triglycerides per turn was approximately 81 kJ/mol, which was sufficient for the stabilization of the complex. Experiments have shown that in a high-temperature frying environment (120 °C), the oil absorption of starch can reach approximately 15%; that is, one triglyceride molecule requires approximately 35 glucose residues for adsorption. In addition, a molecular dynamics simulation was performed to analyzed the interaction between starch molecule and triglyceride molecule. In the simulation system, 500 triglyceride molecules were randomly assigned around a starch molecule containing 50 glucose residues, and the temperature was set at 120 °C. The starch molecule containing 50 glucose residues can typically only effectively complex with one or two triglyceride molecules because of the spatial site-blocking effect. Therefore, when a lipid molecule is complexed with a starch molecule, about 20% of the glucose residues of this starch molecule are directly involved in complexation, whereas about 80% of the glucose residues of this starch molecule are not involved in the binding of lipid.

Please choose the best answer according to the above article:

21. What is the secondary interaction energy of glucose residues with triglycerides per turn? (A) 55 kJ/mol (B) 81 kJ/mol (C) 100 kJ/mol (D) 120 kJ/mol.

22. What is the main function of Glu233 in the catalytic triad of α-amylase? (A) stabilizing the nucleophilic reagent (B) releasing protons and attacking glycosidic oxygen (C) forming a carbon ion intermediate (D) binding starch chains.

23. What type of RS is characterized as retrograded starch? (A) RS-1 (B) RS-2 (C) RS-3 (D) RS-4.

24. What percentage of glucose residues in a starch molecule are directly involved in lipid complexation? (A) 15% (B) 20% (C) 50% (D) 80%.

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25. What is the molecular weight of human α-amylase? (A) 33 kDa (B) 55 kDa (C) 75 kDa (D) 100 kDa.

26. Which factor increases the lipid adsorption rate of starch granules? (A) decrease in temperature (B) increase in water content and temperature (C) presence of α-amylase (D) reduction in granule size.

27. What is Resistant Starch (RS)? (A) a type of starch easily digested in the stomach (B) a starch that resists digestion in the small intestine (C) a starch found only in processed foods (D) a compound that increases blood glucose levels.

28. What is the approximate oil absorption percentage of starch in a high-temperature frying environment? (A) 10% (B) 15% (C) 20% (D) 25%.

29. What structural feature of α-amylase is responsible for starch chain binding? (A) the catalytic triad (B) a cleft 3.5 nm long and 1.5 nm wide (C) flexible loop structures (D) β-folds.

30. Which type of RS is formed by complexation with lipids? (A) RS-1 (B) RS-2 (C) RS-4 (D) RS-5.

Article 5

(Source: *LWT*, 2022 154:112653)

Microorganisms produce hundreds of aroma-active compounds during fermentation process which affect the flavor of the final wine product. The current study explored the enological characteristics of non-Saccharomyces yeast strains and their influence on aroma compound complexity during fermentation process. Forty-two yeast strains were isolated from fruits by the DNA sequencing identification. *Hanseniaspora uvarum* Pi235, *Pichia kluyveri* Pe114, *Saccharomyces cerevisiae* Gr112 and *H. guilliermondii* Ki135 were selected for the following experiments due to the safety considerations, flavor and aroma diversity. Biochemical characteristics results showed that *H. uvarum* Pi235, *P. kluyveri* Pe114 and *S. cerevisiae* Gr112 exhibited different consumption preference in maltose, xylose and glycerol assimilation compared with their BCRC standard strains. Enological characteristics results showed that *S. cerevisiae* Gr112 exhibited better high-temperature thermal tolerance, ethanol tolerance and β-glucosidase activity (90.0 mU/mL). Results of volatile aroma compounds analysis showed that non-*Saccharomyces* yeast strains were good at producing esters, such as ethyl acetate and 2-phenethyl acetate which were 11.8-25.4 and 4.1-15.2 folds higher than that from *S. cerevisiae* Gr112, respectively. Non-*Saccharomyces* yeast strains would provide more diverse flavors in wines. The results will increase the applicability of non-*Saccharomyces* yeasts in the winemaking industry. The results also provide the growth and metabolism characteristics of non-*Saccharomyces* yeast strains which would have more application in fermentation engineering design. Although non-*Saccharomyces* yeasts could not complete the alcohol fermentation due to low ethanol tolerance. Using locally selected yeast strains for co-fermentation between *S. cerevisiae* and non-*Saccharomyces* yeast strains could achieve this purpose and should be worth further investigation.

Please choose the best answer according to the above article:

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31. What's the purpose of this study? (A) the price of wine (B) the production cost of wine (C) the enological characteristics of non-Saccharomyces yeast strains (D) influence on toxic compound complexity during fermentation.

32. How many yeast strains were isolated from fruits by the DNA sequencing identification? (A) forty (B) forty-one (C) forty-two (D) fourteen.

33. Which yeast strain was not mentioned in the article? (A) Pi235 (B) Pe114 (C) Gr112 (D) Ki137.

34. From the results, which strain shows better high-temperature thermal tolerance? (A) *S. cerevisiae* Gr112 (B) *P. kluyveri* Gr112 (C) *H. uvarum* Pi235 (D) *S. cerevisiae* Gr114.

35. β-Glucosidase is a(n) (A) polysaccharide (B) enzyme (C) gene (D) oligosaccharide.

36. non-*Saccharomyces* yeast strains were not good at producing (A) esters (B) ethyl acetate (C) 2-phenethyl acetate (D) ethanol.

37. Which description is NOT TRUE? (A) the results will increase the applicability of non-*Saccharomyces* yeasts in the winemaking industry (B) the results provide the growth and metabolism characteristics of non-*Saccharomyces* yeast strains (C) non-*Saccharomyces* yeasts cannot complete the alcohol fermentation due to high ethanol tolerance (D) Non-*Saccharomyces* yeast strains can provide more diverse flavors in wines.

38. What's the unit of enzyme activity mentioned in this study? (A) mU/mL (B) mU/L (C) U/kg (D) mU/g.

39. Which factor is not considered in this study to choose yeast strains for winemaking? (A) safety considerations (B) growth rate (C) flavor (D) aroma diversity.

40. What's the reason for non-*Saccharomyces* yeasts could not complete the alcohol fermentation? (A) fast growth (B) low ethanol tolerance (C) high ethanol tolerance (D) neutral ethanol tolerance.

Article 6

(Source: *Current Opinion in Food Science*, 2024, 58, 101201)

Currently, liquid chromatography–mass spectrometry (LC-MS) represents the most frequently used technique for analyzing polar and nonpolar metabolites. LC-MS enables the separation and detection of isobars and isomers, reduces ion-suppression effects compared with direct infusion techniques, and separates compounds according to their physicochemical properties. The choice of LC platform can be tuned based on different stationary phase chemistries and compositions of mobile phase solvents and modifiers. In metabolomics studies, reversed-phase liquid chromatography (RPLC) separates polar and medium-polar metabolites, and hydrophilic interaction chromatography (HILIC) separates highly polar to medium-polar metabolites. In lipidomics studies, RPLC separates lipids based on the number of double bonds and the length of fatty acyl chains (lipophilicity), whereas HILIC and normal-phase liquid chromatography separate lipids based on

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their headgroup polarity. Overall, in RPLC, C18 columns dominate, followed by C8 and C30, while in HILIC, different stationary phase chemistries such as silica, aminopropylsilane, alkyl amide, and sulfobetaine groups are used.

Using different mobile phase modifiers has been demonstrated to significantly impact compound retention, peak width and intensity, and the ability to separate isomers. Additionally, it has been suggested that the untargeted methods should not be evaluated purely based on the total number of features, as often presented in research papers. Instead, the evaluation should also consider the performance (*e.g.* compound retention, peak height intensity, and peak width) of common metabolites expected to be detected and annotated using a particular platform (*e.g.* optimal coverage of amino acids using the HILIC platform) and the long-term stability of the retention times. While the impact of organic solvent quality during LC-MS analysis is often overlooked, it has been exemplified with LC-MS-grade isopropanol from different vendors that its quality significantly influences untargeted lipidomic profiling of biological samples.

Derivatization in LC-MS can be beneficial for volatile metabolites, compounds that do not ionize well, or metabolites requiring low detection limits. For instance, analysis of short-chain fatty acids (*e.g.* acetate, propionate, butyrate), usually analyzed using time-consuming gas chromatography–MS-based methods, can be achieved with 3-nitrophenylhydrazine, 2-picolylamine, or O-benzylhydroxylamine, forming hydrazine, amine, and hydroxylamine derivatives of fatty acids. The derivatization of fatty acid esters of hydroxy fatty acids (FAHFAs) using 2-dimethylaminoethylamine has been reported to improve their detection, which is otherwise difficult due to their low concentrations in biological samples. A computer-generated *in silico* library has also been included for their annotation. For carbonyl, carboxyl, and phosphoryl submetabolome, derivatization using 3-nitrophenylhydrazine has been shown to improve sensitivity and coverage.

Ultrahigh-performance LC (UHPLC) with sub-2 μm particles dominates in metabolomics and lipidomics studies due to significant improvements in chromatographic performance. These columns are frequently at 2.1 mm i.d. and compatible with current UHPLC instruments. However, using a 1 mm i.d. microbore column can lead to a 75% reduction in solvent consumption compared with the 2.1 mm i.d. variant, but system modifications are necessary to reduce peak dispersion and prevent clogging in the narrow tubing. Fitz *et al.* showed when comparing conventional 2.1 mm i.d. with micro-setup (1.0 mm i.d.) and nano-setup (0.075 mm i.d.) that micro-LC provided the best compromise between improving signal intensity and metabolome coverage.

LC-MS analysis is adaptable and can be chosen to suit the requirements of food and nutrition research. Integrating untargeted and targeted workflows enables comprehensive metabolite profiling, supporting both hypothesis-generating and hypothesis-driven studies. However, challenges remain in annotating the detected features in complex food matrices and improving the structural elucidation of unknown metabolites. Future advancements will likely focus on enhancing analytical sensitivity in LC-MS analysis, expanding spectral libraries, and integrating advanced computational tools for metabolite identification and interpretation (including artificial intelligence), thereby advancing our understanding of food matrices and biological systems.

Please choose the best answer according to the above article:

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41. Which of the following is NOT the reason why LC-MS has become the mainstream analysis technology for analyzing metabolites? (A) less ion-suppression effect (B) well separation and detection of isobars and isomers (C) effectively separates compounds (D) ability to identify unknown compounds.

42. What does "isobar" mean in this article? (A) atoms of different chemical elements that have the same number of nucleons (B) the same number of atoms of each element, but distinct arrangements of atoms in space (C) a line of equal or constant pressure on a column (D) have the same atomic number, but different nucleon numbers.

43. What is the meaning of "reversed-phase"? (A) polar stationary phase was used (B) hydrophobic stationary phase was used (C) countercurrent of mobile phase (D) the mobile phase is lipophilic.

44. Which compound will be eluted first while RPLC was performed? (A) arachidic acid (B) stearic acid (C) plamitic acid (D) lauric acid.

45. In RPLC, what does C18 stand for? (A) octadecyl (B) eighteen headgroup (C) eighty metabolites (D) eighteen double bonds.

46. What does "untargeted" mean in this article? (A) unnecessary (B) unimportant (C) unexpected (D) unknown.

47. Why derivatization is needed in LC-MS for analyzing volatile metabolites? (A) improve ionization (B) enhance ion suppression (C) augment detection limits (D) boost concentration.

48. What does "i.d." mean in this article? (A) industrial disc (B) intelligent design (C) inside diameter (D) identity function.

49. Why can micro-LC improve signal intensity and metabolome coverage? (A) smaller instrument can accommodate more compounds (B) due to less peak dispersion (C) theoretical plate height can be increased (D) relatively few solvent leads to concentration effect.

50. Which of the following is NOT a technology that LC-MS should improve in the future? (A) enhance sensitivity (B) extend *in silico* library (C) improve computational tools (D) reduce instrument size.

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