MODULE 3. INNOVATIVE PROCESSING OF FISH DISCARDS TO BARF

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Summary

The ability to utilize fish discards to produce Biologically Appropriate Raw Food (BARF) highly depends on the raw materials' quality, stability, safety, and nutritional profile. The present module gives an overview of handling procedures, strategies, and technologies to maintain or improve these parameters. The module is divided into five chapters following the raw material (discarded fish) value chain from capture to a valuable raw material for BARF production. Firstly, the raw materials' properties and stability are discussed briefly. This part discusses nutritional composition, spoilage, and safety issues. Secondly, matters related to catching technology and onboard handling and how to improve these parameters are presented. This section highlights the significance of proper chilling and chilling systems to maintain the quality of the raw materials. Chapters three and four deal with processing concepts increasing the raw materials and the quality and stability of the final product. In chapter three, novel concepts, including the implementation of hurdle principles, are discussed. Commercial technological applications of BARF production are presented in chapter four. Lastly, chapter five introduces packaging concepts for the whole value chain, both discussing bulk concepts potentially used for the distribution of the raw material (discarded fish), as well as consumer-friendly concepts for the distribution of the final BARF product.

1 Fish discards - properties and stability

1.1 Nutritional composition and trace elements

Fish is a source of proteins and healthy lipids but also a unique source of essential nutrients. The proximate composition differs among species, and fatty fish might have large seasonal variations due to the available nutrients and the spawning season. However, fish generally consist of 70-84 % water, 15-24 % protein, 0.1-22 % fat and 1-2 % minerals, and 0.1-1 % carbohydrate [1]. Marine fish species contain a high share of polyunsaturated fatty acids (PUFAs), namely, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), which are essential for animal health and for the proper growth of puppies.

In addition to an excellent distribution of proteins and lipids, fish contains minerals such as iodine, calcium, iron, zinc, selenium, or phosphorus and vitamins B, D, A, and K [2]. Fish protein is regarded as easily digestible and contains all essential amino acids. Although fish consumption has many benefits, it is also a source of chemical and nutraceutical contaminants. Fatty species accumulate heavy metals, pollutants, and nutraceuticals that might be of concern. However, levels are often low, and only in polluted areas and under specific conditions detected values are higher than the limits set by the government. One risk factor highlighted in the Northeast Mediterranean is the content of Arsen (As) in wild captures [3]. Despite some risks observed with fish consumption, they are widely outweighed by benefits [2].

1.2 Fish spoilage: Autolytical, microbial and chemical changes

Quality loss of fish occurs throughout the value chain, from harvesting, processing, distribution, and during storage. The fish raw material is highly perishable due to a high post-mortem pH and a nutrient content supporting microbiological growth. Fish spoilage is based on three mechanisms (1) post-mortem enzymatic autolysis, (2) microbial growth and metabolism, and (3) oxidation reactions [4, 5].

1.2.1 Enzymatic autolysis

At the time of death, the initial loss of fish freshness is caused by autolytic reactions catalysed by endogenous enzymes (Table 1). Post-mortem glycolysis results in the accumulation of lactic acid, which lowers the pH of the muscle. The level also depends on the amount of glycogen in the tissue at the time of death as well as fish species. Stressed fish have a low level and will give a lower pH reduction. Furthermore, autolytic degradation of adenosine triphosphate (ATP), the cell “energy currency”, is one of the first autolytic changes to occur after fish deaths, resulting in a cascade of enzymatic reactions and ATP-degradation products, simplified ATP → adenosine diphosphate (ADP) → adenosine monophosphate (AMP) → inosine monophosphate (IMP) → inosine (HxR) → hypoxanthine (Hx) [6]. Degradation of IMP is related to loss of freshness, and especially Hx is related to fish spoilage.

Autolytic processes also include proteolytic reactions in which proteins are decomposed to peptides and free amino acids. Proteolytic reactions cause softening and textural changes in the fish tissue [7]. Examples of proteases contributing to the deterioration of fish tissue are cathepsins, calpains, and collagenases.

Table 1. Autolytic, microbial, and oxidative changes in chilled fish (modified after Speranza, Racioppo [4], Huss [8], Boziaris and Parlapani [9])

Type of fish spoilage	Causes	Changes
Endogenic (autolysis)	Glycolytic enzymes	Production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle. High-temperature rigor may result in gaping.
	Eenzymes involved in nucleotide breakdown	Loss of fresh fish flavour, gradual production of bitterness with Hypoxanthin (Hx) (later stages)
	Cathepsins	Softening of tissue, making processing difficult or impossible
	Chymotrypsin, trypsin, carboxy peptidases	Autolysis of the visceral cavity in pelagic fish (belly-bursting)
	Calpain	Softening, molt-induced softening in crustaceans
	Collagenases	«Gaping» or fillet softening
	TMAO demethylase	Formaldehyde-induced toughening of frozen gadoid fish
	General endogenic activity	Discoloration (black, yellowish, brownish, paling) and hydrolytic oxidation (accumulation of free fatty acids)
Microbial	*Shewanella* [a]	TMA, hydrogen sulphur compounds, Hx
	Pseudomonas spp.	NH_3, esters, and sulphur compounds but not H_2S
	Photobacterium phosphoreum [b]	TMA, esters, alcohols, ketones, biogenic amines
	Lactic acid bacteria (LAB) and *brochotrix thermosphacta* [c]	Acetic acid, NH_3, tyramine, acetoin, diacetyl, H_2S
	Aeromonas spp., *Vibrio* spp., *Photobacterium* spp., *Enterobacteriaceae* [d]	TMA, sulphur compounds, biogenic amines
	LAB, and *brochotrix thermosphacta* [e]	Acetic acid, NH_3, tyramine, acetoin, diacetyl, sulphur compounds
	Growth of microorganisms, general microbial enzymatic activity	Loss of juiciness, firm texture, discoloration, accumulation of free fatty acids
Chemical	Oxidative rancidity	Rancid flavour and odour, textural changes
	Non-enzymatic oxidation	Discoloration

[a] H_2S-producing *Shewanella*
[b] TMA containing species from seawater at temperatures below 15 °C
[c] Species from warmer waters, particularly species with little or no TMAO
[d] Fresh and lightly preserved products stored at ambient temperatures
[e] Lightly preserved and chilled products

1.2.2 Microbial growth and metabolism

Microorganisms are the main cause of fish spoilage. Fish indigenous microbiota are the naturally occurring microorganisms of fish skin, gills, and digestive tract. The composition of the indigenous microbiota of fresh seafood is affected by geographic origin, water composition and temperature, type of species, and catching methods. Fish from temperate or warm waters contain aerobic or facultative aerobic mesophilic Gram-negative (*Pseudomonas* spp., *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Xanthomonas,* and *Vibrio*) and Gram-positive bacterial species (*Bacillus*, *Corynebacterium*, *Micrococcus,* and *Lactobacillus*). Psychrophilic and psychrotrophic Gram-negative microorganisms, such as *Photobacterium, Shewanella*, *Psychrobacter*, and *Pseudomonas*, predominate in cold water. However, several Gram-positive genera are also commonly reported [9]. The exogenous microbiota results from post-harvest contamination, during on-board handling, and throughout the value chain. Several factors affect the microbial shelf life, including, e.g., capture methods, environmental conditions at the catching ground, fish species, onboard handling and hygiene conditions, and further processing and storage conditions. Temperature control throughout the value chain is critical to inhibit microbial elaboration in the raw material [9].

The microbial metabolism leads to the decomposition of nutrients and the production of specific metabolites causing unpleasant and unacceptable off-flavours and off-smell, discolourisation, slime production, and texture changes (Table 1) [4]. These metabolites are produced by a limited number of microbial species, often known as specific spoilage microorganisms [9].

An unpleasant 'fishy smell' is often caused by trimethylamine (TMA), produced in fish due to Trimethylamine Oxide (TMAO) degradation [10]. TMA is characterised by ammonia-like off-flavours and is produced under anaerobic conditions by specific spoilage bacteria, such as *Shewanella putrefaciens*, *Aeromonas* spp., *Enterobacteriaceae*, *Photobacterium phosphoreum*, *Vibrio* spp., *Micrococcus*, *Acinetobacter*, and *Moraxella*. Other compounds produced causing an unpleasant smell include organic acids, alcohols, aldehydes, amines, ketones, sulfides, ammonia, and hydrogen sulfide (Table 1) [9].

Other biogenic amines are produced through microbial decarboxylation, including histamine, putrescine, cadaverine, spermidine, and spermine. Endogenic enzymes can also produce biogenic amines, but the present microbiota produces the majority. Microorganisms involved in histamine formation are *P. phosphoreum, Enterobacteriaceae,* and *Pseudomonadaceae*.

1.2.3 Oxidation reactions

Fish is a rich source of polyunsaturated fatty acids that, unfortunately, are highly susceptible to degradation processes, such as oxidation. Oxidation processes in fish are accelerated if the raw material is exposed to high temperatures, air (oxygen), and UV light [11]. Furthermore, intensive handling disrupting the muscle membrane can result in oxygen exposure of lipid fraction, and thus oxidation. Oxidation leads to rancid off-flavours. In general, lipid oxidation is more pronounced in minced fish meat than in an intact muscle due to a higher contact surface between lipids and oxygen. Blood removal is strictly linked to fish muscle quality deterioration, especially lipid oxidation. Blood is a pro-oxidant, and the raw fish material can be stabilised by including an on-board operation of bleeding [11]. It is essential to point out that different species and muscle types vary in haemoglobin/myoglobin (the active pro-oxidants in the blood) and lipid content, thus also displaying different susceptibility to be oxidised. Oxidation processes are highly temperature-dependent, and frozen storage at very low temperatures (-30 to -40 °C) can stabilise the raw material.

1.3 Safety challenges

The risk of microbial and chemical contamination of fish raw material is generally considered higher in freshwater and coastal belts than in open seas; however, biological and chemical safety issues vary between regions, habitats, and fish species. Furthermore, it varies according to the production and management practice, including hygienic design and management, in different value chains. The Biosecurity hygiene and EU legislation for the discard fisheries processing and end products are discussed in module 5.

2 Catching technology and onboard handling

Unwanted catches have been one of the main challenges within fisheries and are defined as the portion of the catch that cannot be marketed because they have little or no market value or because of legal requirements [12, 13]. All these commercial and non-commercially resources returned at sea during commercial fishing operations, dead or alive, are called discards [14]. As highlighted by [15], several reasons for discarding exist, including that some individuals might be under the legal minimum conservation reference size, individuals are from species that have low or no market value, they are damaged, or the quota for this species has been reached. It, therefore, cannot be landed from a regulatory perspective. While the practice of discarding can be attributed to social, economic, and legislative reasons, one of the fundamental causes is using unselective fishing technologies coupled with environmental factors affecting species distribution [16]. The degree of discards as a problem is very fishery-dependent, and it has been particularly challenging in mixed-species demersal trawl fisheries, which are currently responsible for most of the discards, at least in the European context [16, 17]. Several countries, including Norway, Iceland, Chile, and New Zealand, have previously established discards bans. The EU landing obligation, also known as the discard ban, was fully implemented in early 2019 by the latest reform of the Common Fisheries Policy (EU 2013, Article 5). One of this ban's aims is to develop and deploy more selective gear. So, from a catching technology perspective, increased selectivity has been the main driver for innovation, reducing the amounts of by-catches and the volume of discarded fish.

Although fishing vessels are intended to catch fish, the reality is that they must also provide adequate conditions for processing and acceptable cold-chain conditions until the fish is landed. The capacity of these systems must also be customised to have the capacity to handle discarded and the rest raw materials in general. The world's total number of fishing vessels is estimated to be 4.4M; more than 80% are less than 12 m long, and industrialised fishing vessels of 24 m or more represent 2% of all motorised fishing vessels [18]. Among these, mostly medium to large commercial scale vessels (12-24 m) going out for mid-distance (around two weeks trips) will have refrigerated seawater tanks and ice-making machines onboard, while only large, industrialised vessels (>24 m) going out for long distances will have the freezing capability. Depending on the species being caught and the processes deployed, these large industrialised vessels will have different blast freezers, plate freezers, refrigerated seawater tanks, and ice-making machines [19].

Freezer trawlers are generally large commercial fishing vessels that operate far from shore with freezing equipment on board. Freezing tunnels (air blast), plate freezers, and freezing tanks (immersion in salt solution and low temperature) are the most used freezing systems in onboard processing vessels. Among these, freezing tunnels might be the best option for discarded fish. Freezing tanks (immersion in salt solution and low temperature) are often used for large whole fish and for pelagic fish used for bait [20].

In a plate freezer, seafood products such as fillets are frozen by contact between two plates with some pressure.

2.1 Hygienic design and cleaning procedures

Hygienic design onboard fishing vessels is essential to ensure the high quality and safety of the raw materials prepared onboard. The skipper and the vessel operator/owner are responsible for ensuring that the vessel is compliant and provides a hygienic fish processing environment. Seafish, in collaboration with the Food Standards Agency (FSA) and industry and enforcement representatives, has produced several hygiene checklists for checking whether fishing vessels comply with minimum hygiene requirements. The hygienic requirements on board fishing vessels are laid out in various regulations and guidelines. For example, the EU has a directive (Directive 93/103/EC) laying down minimum safety and health requirements for work on board fishing vessels. These requirements include regular checks by authorities, technical maintenance of ships and their fittings and equipment, regular cleaning to maintain an appropriate level of hygiene, and suitable training for workers on safety and health on board vessels and accident prevention [21].

Cleaning procedures on fishing vessels are essential to maintain an appropriate level of hygiene and prevent raw material contamination. Some recommended cleaning procedures include cleaning and sanitizing the landing area, all surfaces, and the chilling system (e.g., the wet ice container, the slurry ice system, or the refrigerated seawater (RSW)/ chilled seawater (CSW) tank) daily using proper detergents. The choice of detergents and disinfectants depends on the specific application and must be selected based on the particular needs. For instance, to effectively remove biofilms like those that build up in fish processing areas, chlorinated products such as chlorinated alkaline and sodium hypochlorite solutions or a combination of acidic and alkaline cleaning agents are preferred [22]. It is also essential that all handling equipment is cleaned properly before and after use (including knives, sharpening tools, gaff, and spiking tools or club).

2.2 Onboard chilling and freezing systems

Onboard chilling systems are used in the fish industry to keep the fish fresh and preserve its quality. The onboard chilling process design must be hygienic and handle energy removal proportional to the trawlers' fishing capacity. To optimise the system's chilling process and temperature control, variable fish size and quantity/flow must be considered [23]. Three methods are used in the cold fish chain: storing the fish on ice (brought from land or produced onboard), chilling in RSW tanks, and freezing in blocks in plate freezers. A few vessels also have quick freezers for single frozen fillets [24]. These freezers could also be used for discarded fish stabilisation if a market for this fraction is developed (e.g., the interest among producers of BARF pet food).

For smaller fishing vessels, ice is the most common means of chilling. Other standards are chilled water, ice slurries (of both seawater and freshwater), and RSW. For the full benefits of chilling to be realised, it is essential to maintain chill temperatures throughout the different fish-handling operations [25]. Traditional chilling of a product relies on the fact that no freezing should occur on the surface, resulting in ineffective heat removal. This process becomes even more extensive for larger products due to low thermal conduction and low thermal conductivity, making this process highly energy-consuming and, last but not least, time-consuming [26].

2.2.1 Super and sub-chilling

Super-chilling is the process by which the temperature of the product is lowered to 1-2 °C below the initial freezing point (for fish between -1 and -2 °C) [27, 28]. In contrast, sub-chilling refers to temperatures close to the initial freezing point but not below. Typical temperatures obtained by sub-chilling systems are between -0.5 and -1.5 °C. These definitions tend to be used interchangeably, but super-chilled products typically receive lower temperatures than sub-chilled products. For both procedures, ice formation is found on the surface of the fish, which will conduct heat from the interior part of the fish to the exterior, eventually reaching an equilibrium during storage [29]. This surface ice also acts as a cold reservoir, and the need for external ice is avoided. The ice will also absorb heat from the surroundings keeping the fish at a stable temperature. When super-chilling or sub-chilling the fish, the enzymatic and bacterial activity ceases, and inhibiting microbial growth is one of the most critical factors in increasing the shelf-life of food products. It is reported that the shelf life of fish that has been superchilled can be extended by 1.5-4 times compared to the same food that was merely chilled [27, 30]. Several sub-chilling methods exist, including RSW slurry, partial ice formation, CSW, impingement, and nitrogen freezing. RSW is an ice-water suspension kept at subzero temperature with ice particles surrounded by seawater. The advantages include faster chilling due to faster heat exchange and less physical damage to the fish than flake ice since the ice particles are not directly in contact. The seawater must be salty enough to obtain a low enough temperature. Therefore, excess salt is often added to the RSW system [31]. A more recent technique is the freezing tunnel. The fish is placed on a Teflon-coated aluminum belt which holds a temperature of -8 to -6 °C, and while being transported through the tunnel, cold air is blasting. Another standard super-chilling method is freezing with impingement. This freezer is divided into zones ranging from -30 °C to -40 °C, where the zone closest to the inlet is the warmest, and the area most immediate to the outlet is the coldest. This allows for maximum thermodynamic usage of the refrigerant. The zones also have independent impingement jets, which enable maximum-velocity air to produce maximum heat transfer. Salvadori and Mascheroni [32] reported that processing times in an impingement freezer were lower than the time needed in a conventional belt tunnel freezer, making impingement freezing a promising way of preserving fish products. Superchilling by impingement freezing takes approximately 45-90 seconds. However, this depends on the surface area's ratio to the total sample volume.

Sub- and superchilled conditions have the potential to stabilise discarded fish through different mechanisms, including reduced autolytic activity and hydrolytic oxidation (reduced temperature lowers the action of the endogenous enzymes), as well as reduced growth rates of spoilage bacteria

2.2.2 Freezing technology

Freezing is the process by which the water is converted to ice. In fish, this process slows endogenous enzyme activity that otherwise would have degraded proteins and inhibits microbial growth, eventually extending the shelf life [33]. Fish contains between 60 and 80 % water depending on species and seasonal variations [1]. When temperatures inside the fish reach approximately -1 °C, the water starts to change from liquid to solid [28]. The process can be divided into four phases, as shown in Figure 1. The four phases could be described as; i) sensible heat from the product is removed until the product reaches its initial freezing temperature, ii) cooling continues until the temperature passes the phase-changing temperature, and nucleation of ice crystals starts (the super-cooling phase), iii) latent heat is removed, and ice crystals start to form simultaneously, and last, iv) ice crystal recrystallisation occurs [33]. Freezing is an exothermic

process, and as most of the water turns into ice crystals, the excess heat must be removed from the fish, and depending on what method of freezing is utilised, this process might be slow or fast [28].

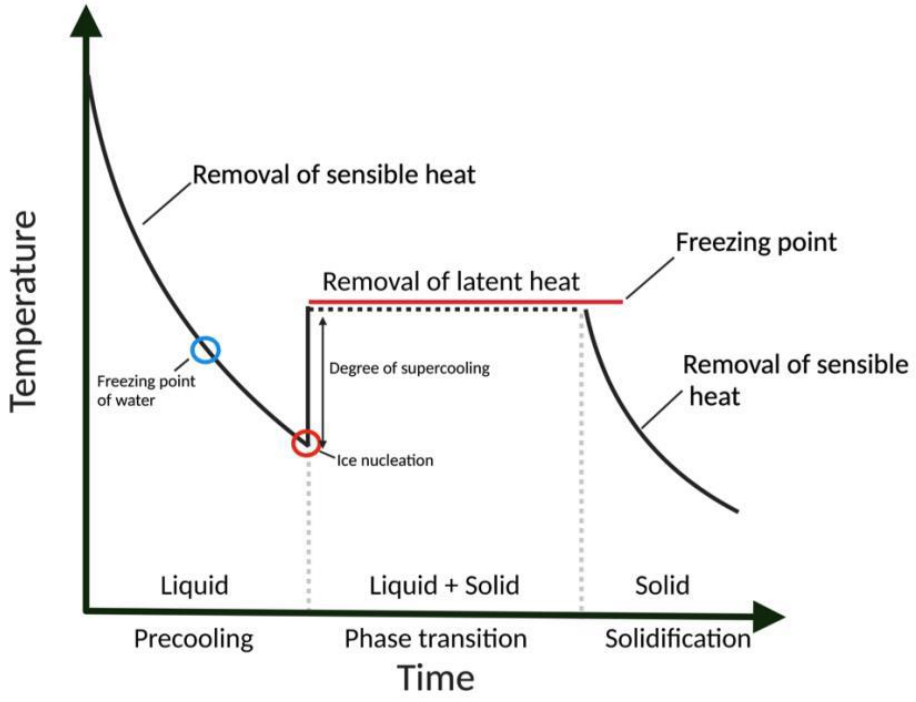

Figure 1. The time-temperature graph of water freezing was modified from Tan, Mei [33].

The freezing rate is critical to the nucleation and formation of ice crystals. A rapid decrease in temperature and fast removal of latent heat supports the growth of numerous small ice crystals [34]. When these criteria are met, small ice crystals form both in the sarcoplasm and the extracellular space, creating opposite forces that result in uniform spacing. This does not cause extensive damage to the muscle cells. Conversely, slow freezing creates more significant structural damage to the muscle structure resulting in drip loss, protein denaturation, and color changes [33]. The ice crystals in the slow freezing form in an irregular pattern. This distinctive feature can be observed in the myofibers and endomysium, disrupting random matters. Recrystallisation is when already formed ice crystals change shape, size, and amount, and when the average size of ice crystals increases, the amount of ice crystals decreases. The driving force is the tendency for all systems to lower the amount of free energy. Recrystallisation results from temperature fluctuations, which are often inevitable during storage and transportation, and as the smaller ice crystals fuse, the larger ice crystals are created. As mentioned, larger ice crystals do more damage to the cellular structures than smaller ice crystals, which is undesirable.

Freezing discarded fish would be beneficial to improve its stability and maximise the potential of its use in BARF. However, several actions must be considered before and during frozen storage to maintain the fish quality. Firstly, discarded fish must be handled on-board as fresh produce, meaning, e.g., it should be bled and chilled sufficiently. Secondly, the fish must be frozen as fast as possible using appropriate freezing technology. If possible, the fish should be either super-chilled or frozen at sea (pre-rigor) by, e.g., using the same freezing systems as for quick frozen fillets. Finally, when frozen, the storage temperature must be kept stable to avoid recrystallisation and loss of quality and nutrients during thawing.

2.2.3 Thawing of frozen discards

Thawing is not practized onshore but is an essential step of sea-frozen fish discards before further processing to BARF. The thawing process is essentially the opposite of freezing; heat is introduced instead of being extracted [35]. When the product is ready to be thawed, the ice crystals develop during the freezing melt. The fish proteins will then try to reabsorb the water if the tissue has experienced minimal destruction. However, suppose the muscle proteins have been denatured due to the freezing. In that case, the ice crystals will melt into the extracellular space creating softer texture, drip loss, and changes in flavor [36]. Drip loss implies nutrient loss as water-soluble proteins remain in the water released during thawing, which results in reduced flesh quality.

The required thawing time depends mainly on the temperature difference between the thawing medium and the product and the characteristics of ice and water. As mentioned, when fish is frozen, a layer of ice forms on the surface, efficiently transferring heat due to the thermal conductivity of ice. When this layer melts, it works as an insulator instead of acting as a conductor, slowing the thawing process immensely. This melted layer will make the thawing process slower and slower as more water is melted. Because of this, thawing is a more complex process to control and predict than freezing [35].

Unwanted protein denaturation due to poor handling before freezing and inadequate freezing, storage, and thawing conditions need attention to use these raw materials as BARF. However, by optimising the specific protocols, there should be a potential to use a frozen raw material for producing BARF.

2.3 Onboard fermentation of fish discards

Bulk fermentation of fish discards by lactic acid bacteria (LAB) is a potential strategy to preserve the raw material before landing and further production to BARF. LAB fermentation in industrial applications is done by spontaneous fermentation or by adding starter cultures. Species of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, and *Leuconostoc* are the most used LAB for commercial starter cultures. From economical, environmental, and animal welfare perspectives, obtaining a stable and predictable microbial population in the feed is of great interest, which can be achieved by developing starter cultures. Fermentation and biopreservation will be further discussed as a strategy for stabilizing BARF products in Chapter 4.1. Fermentation and biopreservation.

2.4 Onboard acidification

Onboard acidification or ensilaging of discarded fish can be a solution to improve the stability of the raw material before BARF production after landing. Fish silage is a liquid product made from whole fish or parts of fish that are liquefied by the action of enzymes in the fish in the presence of an added acid. The enzymes break down fish proteins into smaller soluble units, and the acid helps to speed up their activity while preventing bacterial spoilage. The production of fish silage involves preferably organic acid like formic acid (35 kg/1 tonne of fish) to preserve the fish and then allow the enzymes already present in the fish to liquefy the protein. In further processing into BARF, the ensilage can be a substitute for unprocessed discarded fish or be used as a product it selves. However, specific receipts must be developed based on the product of interest.

3 Innovative processing concepts

3.1 The Hurdle concept

Many traditional or innovative processing technologies may inhibit microbial growth and maintain food and feed quality, including e.g., chilling, salting, adding additives, and biopreservation (Figure 2). These methods can be combined, a concept called hurdle technology [37]. Each of the processing parameters is referred to as a hurdle. The rationale of the hurdle concept, applying multiple antimicrobial factors with moderate intensity, is that synergism may occur by exposing the undesired microorganisms to a series of obstacles resulting in a higher preservative effect than using one factor alone with increased intensity. A synergistic effect occurs if the hurdles hit at det same time, different targets such as microbial cell membranes, DNA, enzymes, etc. An appropriate combination of multiple antimicrobial factors may reduce the intensity of each element, thus decreasing the effect on product quality.

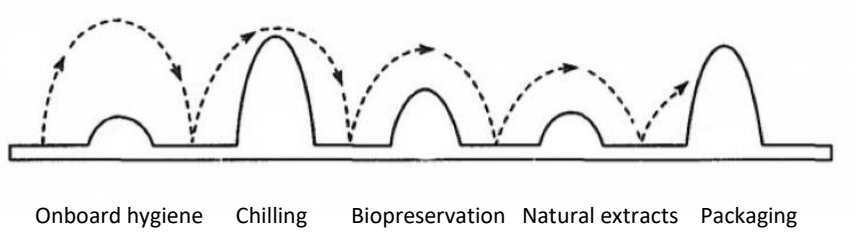

Figure 2. The hurdle concept, giving examples of a potential hurdle strategy for BARF

3.2 Mild processing technologies

Mild processing methods of food products have developed to extend product shelf life and food safety by, partly or totally, inhibiting spoilage and pathogenic microorganisms and/or enzymes while affecting organoleptic attributes, nutritional content, and product characteristics as little as possible [38]. The same approach can be applied to BARF and pet feed production to maintain fresh quality while prolonging product shelf life and inhibiting the potential growth of pathogenic microorganisms.

An overview of different mid-processing methods is given in Figure 3, and the methods of the highest relevance for BARF production are described in the following chapter.

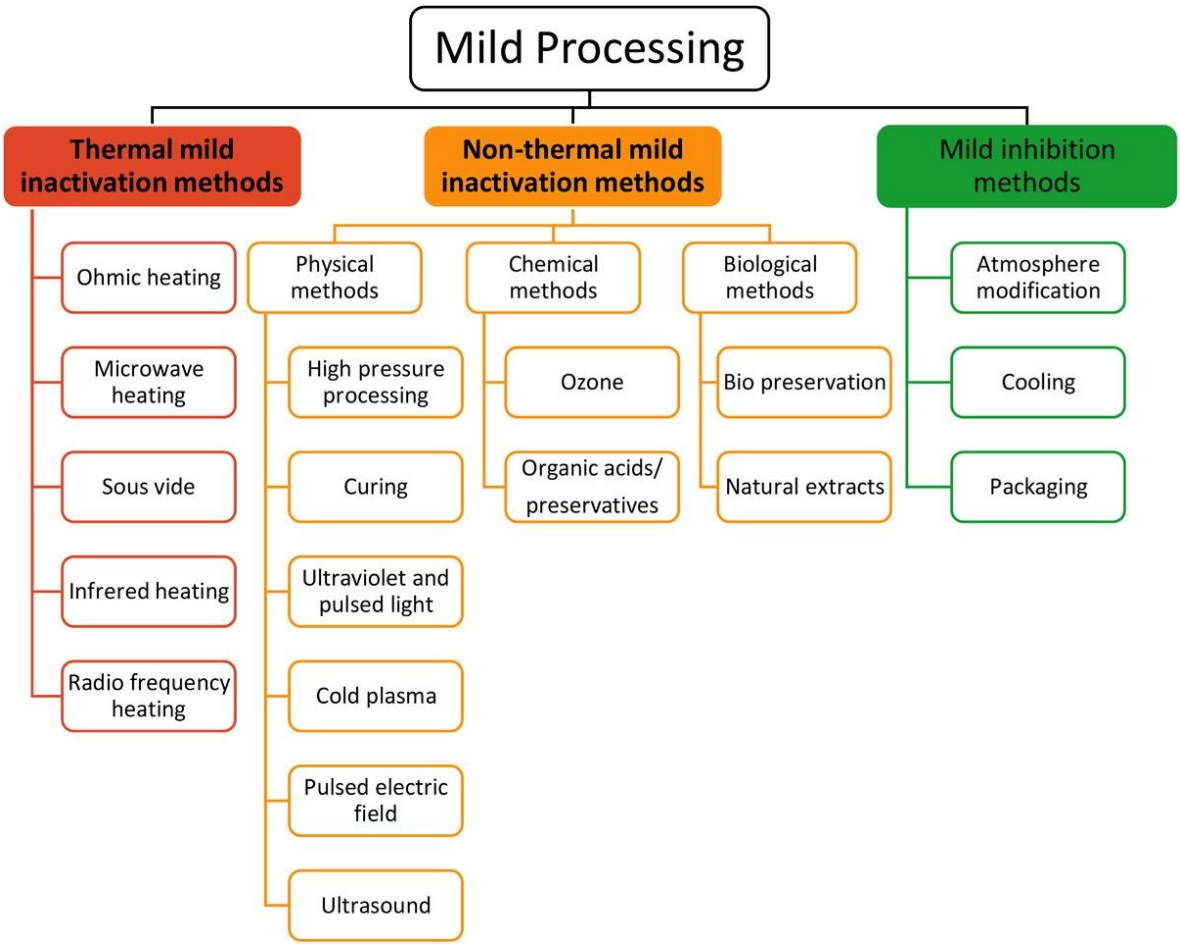

Figure 3. Examples of different approaches to the mild processing of food and feed are separated based on working mechanisms [38].

4 BARF processing technologies

4.1 Fermentation and Biopreservation

Lactic acid bacteria (LAB) are a heterogeneous group of aerotolerant Gram-positive bacteria, and several genera, including *Carnobacterium*, *Enterococcus*, *Lactobacillus,* and *Lactococcus*, are associated with the microbiota of marine fish from cold, temperate and tropical water. Fermentation by LAB is a metabolic process in which these bacteria convert sugars into lactic acid under anaerobic conditions. In foodstuffs, fermentation by LAB is a versatile process that humans have used for centuries to produce a wide range of fermented foods and beverages with distinct flavours and extended shelf life. For preservation purposes, acidification plays a crucial role, as low pH inhibits the growth of spoilage and pathogenic microorganisms. LAB are more acid-tolerant, allowing them to thrive and dominate the microbial ecosystem during fermentation. LAB also produces organic compounds that affect product flavour, texture, and aroma. LAB are also known for their probiotic properties for animals and humans, meaning they can benefit their host by improving their gut health and strengthening their immune system [39].

LAB are generally considered safe (GRAS organisms), i.e., non-toxic and non-pathogenic. LAB are naturally occurring antimicrobial metabolite producers, so they are commonly studied for biopreservation. The concept of biopreservation is inspired by fermentation, except that fermentation involves a substantial transformation of the food matrix, which is usually avoided in biopreservation systems (Figure 4). Biopreservation uses naturally occurring bacteria and their antimicrobial metabolites to control pathogenic and spoilage bacteria in food and feed to extend the product's shelf life [40, 41]. LAB can produce various antimicrobial compounds, such as bacteriocins, hydrogen peroxide, and organic acids. Bacteriocins are proteinaceous antimicrobial substances that target and inhibit bacteria, including pathogenic strains. Hydrogen peroxide and organic acids also possess antimicrobial properties, further contributing to the biopreservation effect [42].

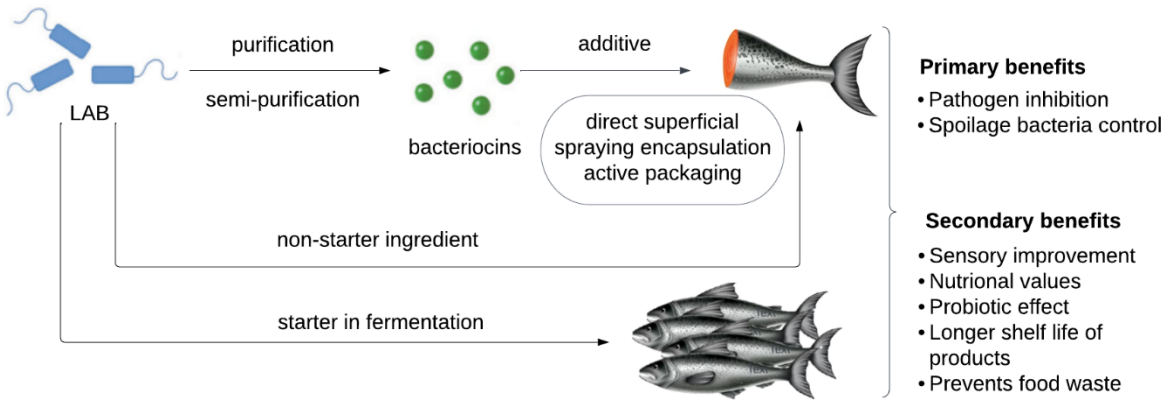

Figure 4. Lactic acid bacteria, bacteriocins, and their application in fermentation and biopreservation of food and feed. Modified from Barcenilla, Ducic [43].

LAB can also outcompete spoilage and pathogenic bacteria for nutrients. They consume the available sugars and convert them into lactic acid, effectively reducing the substrate's nutrient content and limiting the resources available for the growth of undesirable microorganisms. Furthermore, LAB fermentation can alter the microbial ecosystem of the food substrate, favouring the growth of beneficial microorganisms and inhibiting the growth of undesirable ones. LAB can produce substances that create unfavourable conditions for spoilage and pathogenic bacteria, such as bacteriocins that inhibit their growth or metabolic by-products that limit their survival. This microbial shift helps preserve the food and maintain its safety and quality.

Biopreservation can also be used as part of a hurdle technology approach [44], where LAB is combined with other barriers, such as chilling, modified atmosphere packaging, etc., to fight food spoilage and ensure food safety, as illustrated in Figure 2 “the hurdle concept”.

As LAB is a diverse group of bacteria, it is essential to note that different species of lactic acid bacteria can exhibit variations in their fermentation pathways, probiotic properties, and preservative potential. The efficacy can be influenced by the matrix and conditions used. Therefore, in a start-culture-based approach, careful selection of LAB strains and optimisation of production parameters are essential to ensure effective biopreservation and the desired characteristics of the final product. The strains must be

compatible with the raw material matrix, survive adverse conditions during processing and storage, and produce sufficient antimicrobial metabolites against a broad spectrum of pathogenic and spoilage bacteria [42]. The requirements for strains used in biopreservation are illustrated in Figure 5.

Figure 5. Requirement for strains used biopreservation of food and feed products. The figure is modified from Ghanbari, Jami [42].

4.2 Additives

Food and feed additives are chemicals added to prevent microbial, enzymatic, and oxidative deterioration or to improve quality parameters such as texture, taste, and colour. Sugar, salts, and alcohols are additives used for centuries to preserve and enhance food properties. Traditional additives are still utilized in the food industry, but synthetic and natural compounds have developed in later times. Additives can function as preservatives by lowering water activity in the food matrix, thereby reducing the product's microbial and enzymatic activity. They can also adjust pH below or above optimal conditions of microorganisms and mold, work as curing agents to inhibit specific microorganisms, and may function as radical scavengers preventing lipid oxidation. The type of additives to use varies with the product's desired properties. Additives must generally be non-toxic, readable soluble, economical, and practical to access. Inorganic compounds, organic acids, and natural extracts are additives for fishery products that will be addressed in the following sections.

4.2.1 Organic acids

Organic acids are natural compounds with weak acidic properties, giving them an antimicrobial effect, and are either naturally occurring in food or added for flavouring and preservation. Because of their natural occurrence, mainly as by-products of fermentation processes, preservation using organic acids is one of the oldest and most traditional preservation techniques [45]. Today, they have received increased attention due to easy access and not requiring complex processing, making them low-cost and easily

accessible additives. Lactic acid and acetic acids typically occur naturally, while other common organic acids added to processed food are citric-, formic-, propionic-sorbic-, and benzoic acid [46].

The antimicrobial effect is mainly due to the polar side groups of the compounds and the low pH value. Moreover, the acid's efficiency is highly dependent on its pKa value. The polarity makes the acids lipophilic, penetrating the lipid-based cell membrane and dissociating inside the cell. Due to the decreased pH value, important intracellular mechanisms for cell growth, like ATP production and enzymatic activity, will be inhibited [46].

4.2.2 Natural extracts

Natural extracts are derived from plant tissues and contain pigments, polysaccharides, fatty acids, and phenolic compounds, all contributing to antioxidative and antimicrobial effects (Olatunde & Benjakul, 2018).. Antioxidants function as free radical scavengers, which slow down autoxidative processes, which are the most important for lipid oxidation. Antimicrobial effects are related to growth inhibition and lysis of microorganisms. Typical sources of natural extracts include fruit peels (grape, apple, plum), spices (thyme, bay leaf, rosemary), and seaweed (brown algae).

The natural extracts are derived from plant tissues in a two-stage extraction process. In the first stage, the preparation stage, the plant is dried, grinded, or size reduced to separate the desired material from the raw material. The second stage, the extraction stage, usually involves the dissolution of the plant material in organic solvents like ethanol, methanol, acetone, or hexane. Alternative extraction methods such as distillation, supercritical fluid extraction, ultrasound- and microwave-assisted extraction have been introduced as more environment-friendly, time- and cost-efficient extraction methods [47]. Using natural extracts in BARF products will increase their general quality and improve their safety and shelf life.

4.3 Drying and freeze-drying

Drying has been the primary method for long-term fish preservation since humans started to preserve fish. Due to the reduction of water content, drying leads to decreased microbial growth and enzymatic activity, leading to increased product shelf life.

Water activity (a_w) is defined as the water vapor pressure in the raw material divided by the standard vapor pressure of water. The water activity is essential in a drying process because it provides information about the amount of active water present in the product. Most fresh fish products have an a_w of 0.99, meaning that the vapor pressure in the product is 99% of the vapor pressure of pure water. After a drying process, dried fish products should have a_w around 0.6. Generally, $a_w < 0.85$ prevents the growth of all pathogenic microbes and toxins [48].

The range of drying methods can be classified into thermal, osmotic, and mechanical drying based on the watering technique [48]. Thermal drying can further be categorized into air drying, low air environment drying, and modified atmosphere drying. Osmotic drying is performed by adding a solvent, for instance, a salt solution leading to water removal based on concentration differences. In mechanical drying, water is removed by a physical force, like a drum or stamp, giving pressure to the material. For food and feed products, thermal drying is the most common. Tunnel and freeze drying are examples of thermal drying methods for fish products.

4.3.1 Tunnel drying

Tunnel drying is a kind of low airdrying method with continuous product drying. The raw material is on one side of the tunnel dryer, illustrated in Figure 6, and the finished dried product is collected at the other end. Tunnel drying is beneficial due to the easily controllable drying process and the ability to dry large amounts of materials. However, the method is time-consuming and not suitable for thermolabile substances. Stockfish can be produced by tunnel drying. In a study investigating optimal drying conditions of stockfish, it was found that the drying rate of stockfish was affected by the parameters temperature and moisture in the tunnel dryer and, in addition, had smaller fish individuals (1.5 kg) higher drying rate (50 hours) than larger fish individuals (4.5 kg, 75 hours) [49].

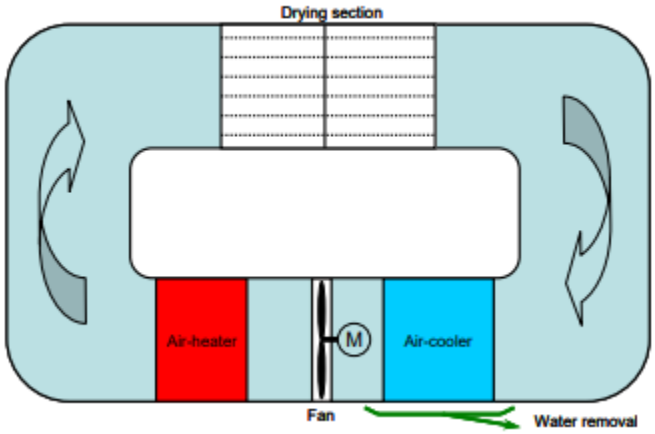

Figure 6. The principle of a tunnel drying system [49].

4.3.2 Freeze drying

In freeze drying, also called lyophilisation, water in the form of ice is removed from a material under low pressure giving almost vacuum and low-temperature conditions [50]. The lack of air and low temperature prevents oxidation and makes heat-sensitive components, such as vitamins, antibiotics, and microbial culture, to be preserved [48]. However, the high energy consumption and time-consuming process make the freeze-drying process expensive for commercial use and limit the method to obtain mainly high-value dried products.

Freeze-dried products are achieved through the three main stages of raw material; 1) freezing, 2) primary drying, and 3) secondary drying [50]. Additionally, the material turns into three phases during the process, shown in a phase diagram in Figure 7.

1. The first stage involves freezing the raw material. This stage is critical for the quality of the end products of the freeze-dried products, as the material must be completely frozen for the subsequent two drying phases. In addition, the freezing rate must be adjusted based on the end product's material type and desired characteristics. During freezing, the water molecules are crystallide into ice crystals of varying size. A higher number of ice crystals with smaller sizes can

be obtained at higher freezing rates; on the contrary, larger and fewer crystals are formed. Smaller ice crystals give low resistance for mass transfer and are beneficial for time-efficient drying.

2. In primary drying, ice is turned into vapor, called ice sublimation. This step has a high drying rate as the free water content is removed. The sublimation occurs from the product's surface during pressure reduction in the freeze dryer. The primary drying stage depends on the freezing chamber's heat supply intensity and pressure.
3. The secondary drying step is called the desorption drying process. Water that is bound to the food matrix is removed. This is the most energy-consuming stage of freeze-drying.

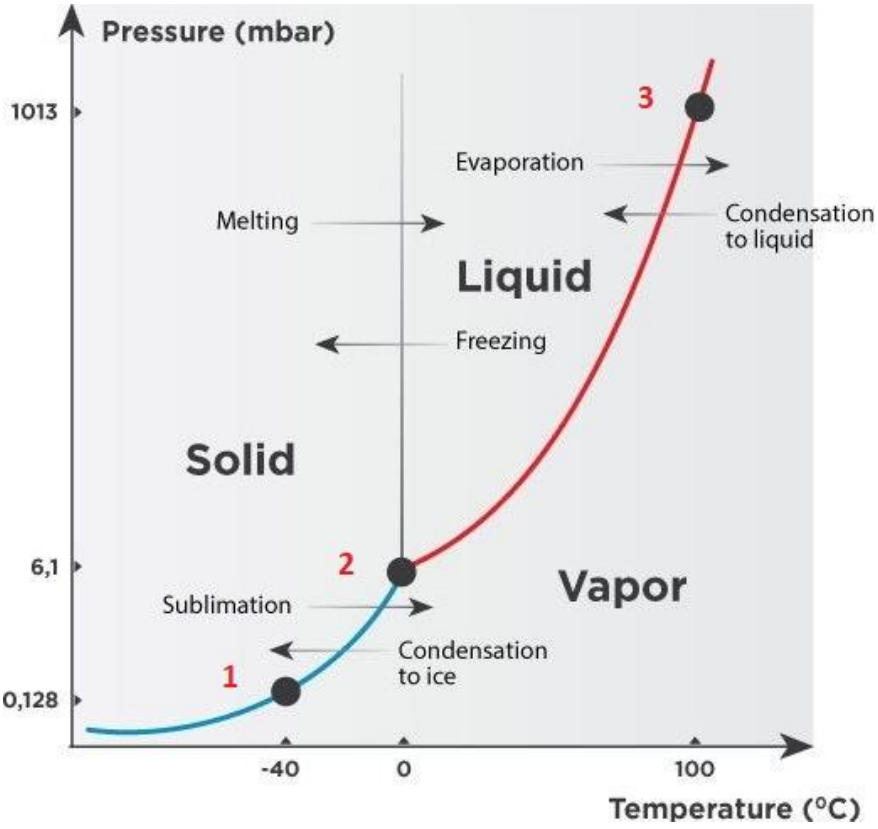

Figure 7. The phase diagram of water shows the principle of sublimation (labogene.com).

5 Packaging

Over the last few years, there has been an increase in the usage of different packaging materials, and there has been a shift in how they are utilized compared to their primary containment function. The shift has been rapid and encompassing towards a usage where the materials have a wide range of properties. These new properties range from the material acting as a barrier against gases, water, and flavor to increasing the product's shelf life and improving aspects related to user convenience [51]. Packaging becomes especially important in seafood, as products made from fish are highly perishable, and good preservation must be maintained for the quality and safety of the product to remain at a desirable level throughout the storage process. The different packaging concepts will affect the quality parameters such

as drip loss, color, odor, rancidity, and shelf life. It is also important to note that the quality of a packaged product will never exceed the quality of the product before it is packaged. Moreover, we must distinguish between needs in the pre-processing value chain and after the BARF is produced.

5.1 Packaging solutions for discarded fish (before it enters the BARF processing plant)

Several packaging strategies must be considered depending on the available technologies for onboard processing. For smaller vessels not having freezing possibilities, proper chilling is mandatory. Bulk packaging in containers (ice slurry or wet ice) or on ice in expanded polystyrene (EPS) boxes might be good alternatives. However, the vessel's capacity must be optimised to handle both the main catch and the discards. For larger vessels, having the opportunity to freeze, other needs occur. The best packaging option for block-frozen discards is corrugated cardboard with a single or double polyethylene (PE) layer as a moisture and gas barrier. Although PE's fair gas barrier properties, it's the most used barrier material combined with cardboard due to its low price and good tear and moisture residence [52]. The packaging materials' moisture residence is also essential for maintaining quality by reducing recrystallisation and rime formation.

Another strategy is to implement active packaging to improve the discards' shelf-life and safety. An alternative for bulk storage of chilled discards is to store the fish either in carbon dioxide (CO_2)-saturated RSW systems or in closed flexible containers using high levels of CO_2. The bacteriostatic effect is strongly correlated to the amount of dissolved CO_2 [53], and pathogens such as *Listeria* spp might be inhibited at high saturation [54]. However, to obtain such high CO_2 levels, other strategies than traditional gas packaging, e.g., Soluble Gas Stabilization (SGS), must be applied [55].

5.2 Packaging solutions for BARF products

BARF products could either be distributed fresh, frozen, or stabilised by drying, affecting the choice of packaging solution. For fresh produce, a solution maintaining excellent shelf-life would be mandatory. This could be gas packaging (referred to as modified atmosphere packaging (MAP). In this packaging concept, the atmosphere in the package is modified to provide more suitable conditions for the product. The principle is to replace air with a different gas mixture (commonly CO_2, N_2, and or O_2), which varies depending on the product, and then seal the package), gas packaging in the form of SGS [55], or a solution including other active packaging strategies. As reviewed by Ahmed, Lin [56], active packaging is a concept where components are incorporated into the packaging material or the package. There is an interaction between component/package, food, and environment, to achieve the desired result for the product. These expected results could enhance the products' shelf life while maintaining the general objective of product quality and safety. Active packaging is divided into two systems: scavenging and releasing. The scavenging systems are typically driven by absorption, absorbing contaminants that may accelerate product spoilage like oxygen, odour, or moisture. Releasing systems typically release or emit certain compounds into the package, like carbon dioxide, antioxidants, or antimicrobials.

For the distribution of frozen BARF products, a vacuum packaging solution stands out as the best option. Vacuum packaging (VP) is a technology where the air in a packet is removed before sealing. The purpose of this packaging technique is to remove oxygen which inhibits the proliferation of aerobic spoilage bacteria, and it will reduce the degree of oxidation of the product. This is done by bringing the packaging material into total surface contact with the packaged product. A significant advantage of VP is that the pack volume is practically the same as the product volume [51] and that it is often regarded as the best

option for frozen products due to the barrier properties of the polyamide (PA)/PE materials often used and the lack of “dead-volume” inside the package.

Packaging of dried products needs even better packaging materials offering excellent gas barrier properties. Dried products, especially those that are freeze-dried, are prone to oxidation, one of the limiting parameters for the shelf life of marine raw materials in general. To cope with this challenge, a vacuum solution often combined with materials with high oxygen barrier properties is used. Some suitable materials that could be applied are typical multilayer solutions consisting of, e.g., PP/EVOH/PP, PA/PE/EVOH/PE, PE/EVOH/PE, PP/EVOH/PE, and PET/MET-PET/PE, where EVOH stands for ethylene vinyl alcohol, PET for polyethylene terephthalate, and MET-PET for metalised (e.g., aluminum)-PET.

Terminology

Source: https://en.wikipedia.org

Autolysis

In biology, autolysis, more commonly known as self-digestion, refers to the destruction of a cell through the action of its enzymes. It may also refer to the digestion of an enzyme by another molecule of the same enzyme.

Endogeny

Endogenous substances and processes originate within a living system, such as an organism, tissue, or cell. In contrast, exogenous substances and processes are those that originate from outside of an organism.

Glycolysis

Glycolysis is the metabolic pathway that converts glucose ($C_6H_{12}O_6$) into pyruvate, and in most organisms, it occurs in the liquid part of cells, the cytosol. The free energy released in this process forms the high-energy molecules adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH). Glycolysis is a sequence of ten reactions catalyzed by endogenous enzymes.

Metabolism

Metabolism is the set of life-sustaining chemical reactions in organisms. The three main functions of metabolism are converting the energy in food to the energy available to run cellular processes; converting food to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and eliminating metabolic wastes.

Exothermic process

In thermodynamics, an exothermic process is a thermodynamic process or reaction that releases energy from the system to its surroundings, usually in the form of heat but also a form of light (e.g., a spark, flame, or flash), electricity (e.g., a battery), or sound (e.g., explosion heard when burning hydrogen).

Fermentation

Fermentation is a metabolic process that produces chemical changes in organic substances through the action of enzymes. In biochemistry, it is narrowly defined as energy extraction from carbohydrates without oxygen. In food production, it may more broadly refer to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage.

Biopreservation

Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. The biopreservation of food, especially utilizing lactic acid bacteria (LAB) that are inhibitory to food spoilage microbes, has been practiced since early ages, at first unconsciously but eventually with an increasingly robust scientific foundation.

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