

REVIEW ARTICLE

Emerging ingredients for clean label products and food safety

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Abstract

Emerging markets comprise different healthy food products, such as functional foods including grains, fruits, and vegetables; foods for people with dietary restrictions; foods with clean labels, plant-based and organic ingredients, as well as ingredient options to replace sodium, sugars, fats, as well as alternatives to synthetic nitrite. The variety of ingredients with potential applications in these products raises relevant safety issues due to the lack of studies about possible biological contaminants, and nutritional, chemical, microbiological and allergen concerns. Besides, certain ingredients may present the potential for allergenicity, intolerance, or other immunological reactions, such as celiac disease. Derivatives of alternative plant-based proteins are examples of these risks and should be assessed for their allergenic potential and specific microbial load. Food safety refers to risk management, based on control measures established throughout the production chain, not limited to quality tests on the final product. This review addresses the main categories of food recognized as healthy by consumers, from a food safety point of view, pointing out the potential risks and management alternatives available for this new market of clean label products.

Keywords: Food industry; Clean label; Healthiness; Technological innovation.

Highlights

- The search for healthy food is a growing trend and presents major challenges for the clean label market
- Novel ingredients raise safety questions regarding the presence of biological contaminants, anti-nutrients and health risks
- The clean label market needs to adjust to the Brazilian food system to guarantee food safety



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1 Introduction

The selection of food for a meal is increasingly responsible and complex, as it directly affects the health and well-being of the consumer, as well as the impact on the environment and animal welfare (Michel et al., 2021).

After the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic was declared by the World Health Organization (WHO) as a public health emergency of international concern (World Health Organization, 2020), there was an increase in consumers' perceptions of health habits. As a result, there has been an increase in the consumption of healthy foods, which is expected to rise in the long term (International Food Information Council, 2020). The food industry has also undergone changes in its production system to meet the demands of food safety, as well as food security and sustainability (Galanakis, 2020).

The Clean Label movement is one of the biggest trends of the decade (Shelke, 2020), which has brought challenges for the food industry to meet the demand for food that is closer to natural, with few additives, less caloric ingredients, less processing, and good stability throughout the storage (Nachay, 2017b; Asioli et al., 2017; Shelke, 2020). From the point of view of consumers, such products can be healthier and pose fewer risks to their health (Asioli et al., 2017). Despite the controversies and lack of clear definitions of the term clean label, it is known that food processing using appropriate technologies plays an important role in controlling undesirable reactions and physical, chemical, and microbiological contaminants, as well as extending the shelf life and maintaining the nutritional properties of the product.

The possible changes in stability and technological functionality required for the production of foods labeled as healthy may require reformulation and process adjustments, as well as changes in packaging, when compared to traditional counterparts, aimed to guarantee the nutritional and microbiological safety of these products. Examples include reduced-calorie products, cured meats using nitrite substitutes (Flores & Toldrá, 2021), and plant-based products that have a very different microbial flora (Fraser et al., 2018; Geeraerts et al., 2020).

Certain nutritional ingredients used in human food have the potential to cause reactions or health problems in susceptible individuals, whether due to allergy, intolerance, or other immunological reactions, such as celiac disease. Appropriate labeling is required for foods destined for these high-value-added niche markets, in addition to the traceability of the entire production chain, with the implementation of protocols or the use of intelligent technologies to guarantee product safety.

This review looks at the main categories of food recognized as healthy by consumers, from a food safety point of view, pointing out the potential risks and management alternatives available for this market of novel healthy products.

2 Theoretical references

2.1 Dietary restrictions & healthiness

The population growth and the consequent need for new sources of nutritious food, and diet-related diseases (allergies and intolerance) are two major challenges of the 21st century, impairing the implementation of novel food alternatives (Ogrodowczyk et al., 2021).

Allergic reactions caused by food represent a serious health hazard (Abdelmoteleb et al., 2021), requiring traceability and strict control of food allergens throughout the production chain to avoid cross-contamination (Ortiz, 2020). The use of technology, such as big data, can help manage potential risks through sensor monitoring (Misra et al., 2020).

New foods such as genetically modified agricultural products and those derived from alternative plant-based proteins are a source of risk and should therefore be assessed for their potential allergenicity. Some countries have started to regulate the cross-reactivity of plant ingredients, with allergen databases created from whole genome analysis or proteome bioinformatics. In this context, cross-referencing data (in silico methodology) allow assessing the risk potential of novel foods before they are marketed (Abdelmoteleb et al., 2021).

The importance of establishing guidelines for the risk assessment of biotechnology-derived plants (Genetically Modified Organisms (GMO) plants) arises from the possibility of expressing different heterologous allergenic proteins (World Health Organization, 2003).

The consumption of plant-based proteins is a recent trend in Brazil, with a growing consumer demand, both due to dietary restrictions resulting from allergies to animal proteins (egg, milk, fish, etc.), food intolerance (lactose, casein and others) and the need to reduce dietary cholesterol (Rosenfeld & Burrow, 2017; Montemurro et al., 2021), as well as flavor diversification, religious and economic reasons (Hoffman et al., 2013), or eating habits (vegan, vegetarian, and flexitarian diets).

Plant-based food consumers can be classified as those looking for products that are similar to meat from the sensory point of view or those looking for a substitute with a similar nutritional profile to meat (Hoek et al., 2011). However, many consumers are concerned about providing substitutes with similar nutritional quality to those of animal origin (milk, meat, and eggs), with adequate protein content, balanced amino acid profile, and digestibility (Vogelsang-O'Dwyera et al., 2021).

A diet that reduces or excludes meat and dairy products can have unintended nutritional effects, due to the selection of foods with lower nutritional density or foods that require preparation with oil or salt. Except for the traditional vegan diet, all diets with traditional plant-based substitutes meet the daily requirements for calcium, potassium, magnesium, phosphorus, zinc, iron, and vitamin B12 and are lower in saturated fat, sodium, and sugar when compared to the reference diet. Many plant-based foods do not contain all the essential amino acids and are called “incomplete” protein sources (Tso & Forde, 2021).

The recent innovation in the plant-based products sector has focused on organoleptic properties (texture, flavor, and appearance) and formats (nuggets and burgers), rather than innovative ways of increasing nutrients in plant derivatives to ensure a balanced nutritional profile similar to that of animal products (Tso & Forde, 2021).

Plant protein sources have anti-nutritional factors with adverse effects on digestion and nutrient absorption, also known to cause abdominal discomfort. The main compounds are saponins, phytic acid, alkaloids, certain oligosaccharides, protease inhibitors, cyanogenic glycosides, glucosinolates, and tannins. However, these compounds have received considerable interest due to the biological activities that can be beneficial to humans, such as antioxidant capacity (Pihlanto et al., 2017), thus it is noticeable that certain content in the food can bring health benefits.

During the traditional processing or preparation of vegetables, there is a reduction in anti-nutritional factors, mainly after heat treatment. However, the growing demand for plant-based products has led to the use of technologies for concentrating proteins by physical methods, without the use of chemical reagents, such as dry fractionation. This process concentrates the proteins, but also the anti-nutritional factors, requiring additional treatments to reduce them to safe levels, using technological processes such as fermentation, thermoplastic extrusion, microwaves, and high pressure (De Angelis et al., 2021).

2.2 Naturally functional foods: grains, fruits, and vegetables

Cereal grains have unique phytochemicals that complement those of fruit and vegetables when combined in a diet (Liu, 2007). In addition, grains, fruits, and vegetables are recognized as a source of dietary fiber (Food and Agriculture Organization, 2020).

Bakery products are highly consumed worldwide, and the inclusion of phytochemicals has been encouraged due to their health benefits (Cappelli & Cini, 2021). In Brazil, regulations state that whole grain flour must contain the three fractions of the grain (starchy endosperm, bran, and germ) in the typical proportion of the intact grain, and losses of up to 2% of the grain or 10% of the bran are allowed (Brasil, 2021). The importance of maintaining the grain fractions is due to the health benefits arising from the interaction between the micro and macronutrients in the grains (Zhang et al., 2021).

Ensuring safety against the presence of biological (pathogenic microorganisms, toxigenic, etc.), chemical (mycotoxins, pesticide residues, metals, etc.) and physical (insect fragments, foreign materials, etc.) contaminants is the challenge in the production of whole grain products (Tibola et al., 2009). Brazil's hot and humid climate favors the development of toxigenic fungi in grains, requiring appropriate pre- and post-harvest procedures (moisture control, segregation of contaminated grains), transportation, storage, and processing (Tibola et al., 2009). Preventing contamination by mycotoxins is fundamental, as they are stable compounds that tend to remain in the food when present. Various physical, chemical, and biological techniques, as well as processing conditions, can be used to reduce contamination and partially control the production of mycotoxins (Daou et al., 2021).

The use of big data technologies has the potential to help reduce the incidence of contamination by toxigenic fungi by combining agricultural and environmental data (weather reports or satellite images) to predict dangers in agriculture (Deng et al., 2021). Research has shown that climate change can affect the development of cereal crops and the occurrence of mycotoxins (van der Fels-Klerx et al., 2012). These authors assessed the impacts of climate change on the occurrence of deoxynivalenol (DON) in wheat grown in northwestern Europe up to 2040, considering the combined effects of changes in climate and wheat phenology. Similarly, the use of predictive models on a local scale can be effective for monitoring other risks, allowing management of the best time for planting and harvesting, segregating batches with higher levels of contamination, or taking other sanitary measures to ensure food safety.

In Brazil, losses of up to 2% of the grain or 10% of the bran are allowed in the regulation of whole grain products for sanitary reasons. The use of procedures such as peeling or pearling allows for the reduction or elimination of chemical and microbiological contaminants present on the outer portion of the grain. The benefits of the phytochemical compounds of the grains depend on the processing methods and conditions, which can influence the nutritional components and the bioactive compounds (Zhang et al., 2021).

Several initiatives have been carried out around the world aimed at including fruit and vegetables in their various forms (fresh, dehydrated, frozen, freeze-dried, and in the form of juices) in diets due to their health benefits. One of the innovative initiatives to increase the consumption of these foods is to obtain isolated ingredients from whole fruit or vegetables (including peels, seeds, and leaves) and use them to enrich other types of products (Salehi & Aghajanzadeh, 2020). Special emphasis has been placed on plant extracts as a clean label ingredient to impart color, aroma, and flavor, which is an emerging tendency in the natural beverages and flavors segment. Although the dosage of pesticide residues is one of the concerns of food manufacturers, these contaminants have not been detected in extracts, probably because most pesticides are oil-soluble, while the plant extracts are water-soluble.

The initial aim of the clean label concept was to encourage consumers to consider plant-based foods as healthy products. However, the concept has expanded to include unprocessed or minimally processed foods (Asioli et al., 2017). This concept has evolved over the years (Roobab et al., 2021), aiming to eliminate or reduce artificial ingredients or replace them with natural ingredients that provide specific functionality.

The consumer's demand for organic products has also increased worldwide. Organic farming is a production system that maintains the health of soils, ecosystems, and people. It relies on ecological processes, biodiversity, and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic farming is based on principles of health, ecology, justice, and care (Asioli et al., 2017). Consumers believe that organic food products are healthier and safer. According to several studies (Lin et al., 2021; Lima & Vianello, 2011; Das et al., 2020), these foods can be considered healthier, with lower levels of pesticides, hormones, and nitrates and higher levels of vitamins. However, there is no conclusive data about the distinct nutritional and functional composition of organic vegetables (Lima & Vianello, 2011), despite their higher levels of antioxidant compounds, especially phenolic compounds. Antioxidants are produced as a form of natural plant protection, which may explain their higher concentration in organic vegetables. However, studies should take into account the various factors that can alter the nutritional quality of a food, such as

harvest time, climate, soil characteristics, environmental conditions, and cooking processes (Trewavas, 2004).

Initiatives to use methodologies for monitoring and predicting pesticide levels in the field through Big Data and Artificial Intelligence (AI) are still in the early stages but are expected to increase in the coming years, especially due to the trend towards the use of smart technology in agriculture or Agriculture 4.0 (Misra et al., 2020).

2.3 Health impact of clean label ingredients

The absence of a legal definition and specific regulations impairs the interpretation of clean labels, particularly about the terms “artificial” and “natural”, for both consumers and manufacturers. Evidence suggests that unknown ingredients or those with hard-to-pronounce names are perceived as more harmful (Moskowitz et al., 2012). Synthetic chemical additives influence the public’s perception of food risk, which correlates positively with a preference for more natural products (Maruyama et al., 2021). Research suggests that consumers interested in clean label foods look for minimally processed products with fewer additives and familiar ingredients (Cargill, 2017).

The manufacture of stable products with clean label characteristics is one of the current challenges facing the food industry (Nachay, 2017b), as “stable” and “natural” can be considered paradoxical terms in terms of technological aspects, since natural or minimally processed foods are usually consumed quickly or have a short shelf life. However, processing is responsible for stability, allowing for an extended shelf life of the product, with a consequent reduction in post-harvest losses, prolonged conservation, quality, safety, availability, and optimization of nutrients (Rego et al., 2020).

A lot of research has been carried out on alternatives to replace critical ingredients such as colorings, flavorings, preservatives, and other artificial additives. The market has been offering commercial products with a clean label trend, thus appropriate processes for reducing or replacing ingredients are required to guarantee product safety. The process of acidification or the use of naturally acidic ingredients, followed by pasteurization and aseptic filling can guarantee the stability of products such as juices at room temperature, without the addition of preservatives, as well as sterilized and aseptically filled products. By definition, commercial sterilization promotes the destruction of pathogenic micro-organisms, spoilers, and enzymes, before or after packaging, aimed to ensure sanitation and preservation, with minimal sensory and nutritional losses, for some time, without the need for additional preservation methods, promoting stability at room temperature, thus allowing the production of a clean label product.

2.4 Natural fermentation with sourdough

In the bakery sector, sourdough, levain, or natural yeast is one of the biotechnological ingredients that meets the consumer requirements for healthy foods. The products of lactic acid bacteria (LAB) metabolism provide multiple benefits, such as antimicrobial effects and improved nutritional, technological, sensory, and functional properties. Enzymes, organic acids, exopolysaccharides (EPS), and antimicrobial compounds stand out among the beneficial metabolites of LAB (Plessas, 2021). The lactic fermentation used to obtain sourdough is an ancient technology, but the global market has been looking for its stabilization for industrial use, through drying methods (spray dryer, freeze-drying, or immobilization), without losing the beneficial characteristics of the fresh product. For gluten-free bakery products, sourdough is also a clean label alternative (Montemurro et al., 2021).

Bread making using sourdough is more common in specialized bakeries, which use natural yeast in rustic bread, Italian bread, ciabattas, focaccia, and others. Industrial products include panettone and colombas, and more recently in Brazil industrial bread versions (traditional or whole grain loaf, *bisnaguinha*, etc.), toast, gluten-free bread, and cracker cookies. However, products made from sourdough need to be regulated in

Brazil to standardize the product quality, once only the state of Santa Catarina, through Decree 31455/87 has established the use of selected yeasts of proven purity by an official laboratory and prohibited the use of sourdough starters (Santa Catarina, 1987).

The regulations in France (Décret n. 93-1074) and Spain (Royal Decree 308/2019) set standards of identity and quality for products made from sourdough, allowing their use in an active state, dehydrated form, and inactive form (France, 2019; España, 2019). Both countries allow the use of a maximum of 0.2% (flour basis) of commercial yeast in the final stage of bread made from sourdough.

2.5 Plant extracts

To meet the consumers' demand for a healthier and natural diet, plant extracts are revolutionizing the food and beverage industry, which are incorporated into a wide variety of products. Botanical extracts are obtained from plants, roots, flowers, and fruits, and have been used throughout history in various cultures for medicinal and culinary purposes. However, their recent popularity is related to the growing desire to consume more natural food products with health benefits (Aditivos Ingredientes, 2023).

One of the main aspects of this trend is the diversity of options. Plant extracts can include a wide range of ingredients, such as turmeric, ginger, chamomile, lavender, peppermint, and green tea, among others. Each one provides unique flavors and functional properties that can be incorporated into a variety of products, from energy drinks to yogurts and desserts (Aditivos Ingredientes, 2023).

Plant extracts have potential antimicrobial efficacy and have been applied to various products, such as bakery products, meat, and confectionery, among others (Rota et al., 2008; Gonçalves et al., 2017; Aziz & Karboune, 2018). In extracts obtained from spices, the antimicrobial effect is due to the presence of phenolic compounds, terpenes, and coumarin, among others, which alter cell permeability and interfere with the transport of electrons in the membrane, altering the metabolism of nutrients and enzyme activity. Among these extracts, the most commonly used in the bakery and meat industries are those of rosemary, clove, sage, oregano, and green tea. The phenolic compounds present in high quantities in these extracts act as antioxidants and antimicrobials, prevent lipid oxidation, and contribute to the development of color and flavor in meat products, as well as anticarcinogenic and antiviral effects.

Plant extracts have stood out as an excellent alternative to replace synthetic antioxidants, as they can improve the oxidative stability of food products, which can lead to an increase in shelf life. In addition, these compounds can act as antifungals and inhibitors of mycotoxin production, such as aflatoxin, by acting to regulate lipid peroxidation, inhibiting the formation of peroxides and consequent oxidative stress that is related to aflatoxin biosynthesis (Food Ingredients Brasil, 2016).

The standardization of plant-based ingredients is an important issue for food manufacturers and consumers. There are several active compounds in plants that can act cumulatively or synergistically. Another key issue for plant food additives is plant safety. Examples of herbs with adverse effects include chaparral, ephedra, blue cohosh (*Caulophyllum thalictroides* (L.) Michx.), and Yohimbe among others. Other safety concerns include potential interactions with other ingredients. In general, the food industry uses extracts with all the molecules extracted, without isolating any specific molecules. The toxicity of extracts is another worrying aspect and their safety must be investigated (Aditivos e Ingredientes, 2012). In addition, several studies are needed to assess the effects of food processing on the active compounds (Moura et al., 2019a, 2019b).

Plants can accumulate several active ingredients in some of their parts and their therapeutic properties can be used to treat various diseases. Medicinal plants can be used for therapeutic, culinary or cosmetic purposes and are subject to primary and secondary processing, from which they are used in different ways: in their natural state (fragments, powders, etc.), infusions, decoction, maceration or phytotherapeutic products for internal or external use in the form of extracts, tinctures, syrups, aromatics, oils, etc., requiring assessment regarding toxicity and health risks of consuming these plants in different forms (Barbeş et al., 2023).

The use of plant extracts in agriculture has potential demonstrated by the high number of publications in the last ten years, with emphasis on the years 2020, 2021 and 2022, with Brazil being the country with the largest production of works focused on this area of study. The themes of control of phytopathogenic microorganisms, insect control, biostimulant effect, induction of resistance and herbicide effect have been the main themes explored for use in agriculture (Carvalho et al., 2022).

Plant extracts and bioactive dietary components play a significant role in the maintenance of human health and well-being, with the potential to modulate risk factors and control symptoms of a large number of common disorders, such as memory impairment, respiratory diseases, gastrointestinal disorders, metabolic disorders and related pathologies for the oral cavity (Ullah et al., 2021). Time is needed to expand the literature data covering the efficacy and safety of infant supplementation with botanical ingredients, extracts and bioactive food components, especially about dosage, and method of ingestion to avoid interactions with medications and other foods or food components.

Based on plant species, the recovery yield, quality and nature of phytochemicals vary from plant to plant and are also influenced by extraction method, extraction time, extraction temperature, solvent: water ratio and solvent polarity. Several extraction methods prioritize economic efficiency, simplicity, respect for the environment and obtaining high extraction yields (Thangaiyah et al., 2024). Some methodologies for extracting phenolic compounds involve new technologies, such as pulsed electric field (PEF), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE); MAE uses green solvents (water and ethanol), which are more environmentally friendly and have short extraction times (Campos-González et al., 2024).

The use of plant extracts in the food industry has also grown due to healthiness and naturalness trends (Aditivos Ingredientes, 2023). The application of hibiscus calyx extract, known to have antioxidant and coloring capacity, in processed products such as jelly candies and yogurts (Moura et al., 2019a, 2019b), showed the antioxidant effect of the presence of phenolic compounds, especially when the extract is microencapsulated, in addition to being a good natural pink colorant. The application of yerba mate extract in fruit bars (Budin, 2022) and sausages (Wensing, 2024) also shows the antioxidant and antimicrobial capacity of this extract.

The innovation in the food industry has led to the creation of products that offer a unique sensory experience by combining botanical extracts to form unique and intriguing flavors that appeal to the most demanding palates. This trend is changing the food industry, offering consumers a wide variety of choices. The evolution of research and innovation can lead to the emergence of new botanical ingredients, further expanding the possibilities for creating products that meet consumer demands for unique flavors and health benefits (Aditivos Ingredientes, 2023).

2.6 Natural colorings

Colorings are defined as substances that modify the perceived color of objects or impart color to other colorless objects. On the other hand, the term “natural” means “present or produced by nature; not artificial or man-made” and “Not altered, treated or disguised.” According to Mohamad et al. (2019), natural colorings are substances produced by nature (obtained from plants, animals or minerals) that modify the perceived color of objects, or transmit color or otherwise colorless objects.

Nowadays, most synthetic colorings are derived from toxic petroleum products and natural colorings are derived from plants, minerals and animals. Artificial colorings are easier to produce and can be introduced onto the market at cheaper prices. Furthermore, in small quantities, they can provide the desirable color. Some examples are amaranth, erythrosine red; twilight yellow; indigotine blue and rapid green. The joint FAO/WHO committee of experts on food additives, JECFA (“Joint Expert Committee on Food Additives”), at an international level, recommends that each country periodically check the total consumption of each additive, based on diet studies, to ensure that the total intake of the additive does not exceed the ADI (Acceptable Daily Intake).

Artificial colorings, also known as food colorings, contain multiple chemical compounds. Most of these chemical compounds are produced from highly toxic sources and can cause numerous human diseases,

disorders and mutations. (Ahmed et al., 2021). It is known that the presence of basic functional groups, the NH_2 amine function, for example, is essential for the carcinogenic activity of colorings. Thus, an attempt was made to eliminate these harmful properties of azo colorings, introducing carboxylic (COOH) or sulfonated (SO_3H) groups into their structures in place of these functional amine groups. In addition to reducing their carcinogenic action, this made them water-soluble, which allowed them to be quickly eliminated, while the original matrix was lipophilic, retaining them in the body for a long time (Henrique, 2021).

On the other hand, the use of natural colorings is reinforced by scientific discoveries about the health benefits of various groups of pigments such as anthocyanins and carotenoids. For example: *Hibiscus sp* which contains up to 2.5% (dry weight) anthocyanin has historically been used in reducing liver dysfunction and hypertension (Stănciuc et al., 2017; Mohamad et al., 2019). Furthermore, natural colorings are preferred by consumers when compared to artificial colors due to safety concerns (Mohamad et al., 2019).

Color is present throughout nature in fruits, vegetables, seeds and roots and can be present in the daily diet in large quantities in the form of pigments, especially anthocyanins, carotenoids and betalain (Mohamad et al., 2019).

Anthocyanins (Figure 1) are common in higher plants but are absent in some lower plants and algae. The anthocyanin structure exhibits optimal performance and color variety (e.g., red, purple, and blue) in flowers, fruits, leaves, and storage organs of higher plants. They are soluble in water, which facilitates their incorporation into aqueous systems.

According to Technical Regulations on the recommended daily intake of proteins, vitamins and minerals (Brasil, 2006), there is no recommendation for the consumption of anthocyanins as a source of antioxidants, however, as a comparison factor, the recommended daily intake of vitamin C for adults is 45 mg/day. In Europe, the anthocyanin intake ranges from 18.4 (Spain) to 44.1 mg/day (Italy) in women and 19.8 (the Netherlands) to 64.9 mg/day (Italy) in men. However, 50 mg/day of daily anthocyanin intake in China is recommended (Gonçalves et al., 2021). Also, according to an exposure estimate, for an average adult weight of 70 kg, anthocyanin intake of 49-133 mg/day could be well-tolerated (Saini et al., 2024)

Anthocyanins extracts are extensively used as natural colorants in diverse foods, including confectioneries, preserves such as jams and jellies, and sausages. They are also prominently utilized in several beverages, ranging from dairy products like yogurts to fruit juices. This widespread utilization underscores the versatility and significance of anthocyanins in the food and beverage industry (Saini et al., 2024).

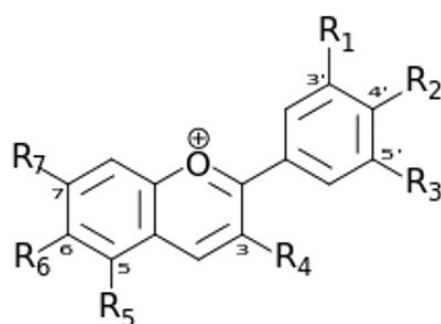


Figure 1. Structure of the flavylium ion characteristic of anthocyanins. Where R_1 , R_2 , R_3 , R_4 , R_5 , R_6 and R_7 can be hydrogen atoms, hydroxyl (OH) or methoxy (OMe) groups.

Source: Moura et al. (2018).

Carotenoids (Figure 2) are widely distributed in nature. Lycopene ($\text{C}_{40}\text{H}_{56}$) is considered the first colored carotenoid in the biosynthesis of many other natural carotenoids and is linear. Carotenoids are characterized by being fat-soluble and having oxidizable molecules. Its chemical structure is tetraterpenoids, made up of forty carbon atoms, having the property of absorbing visible light at different wavelengths.

Lycopene currently appears as one of the most potent antioxidants, being suggested for the prevention of carcinogenesis and atherogenesis by protecting molecules such as lipids, low-density lipoproteins (LDL), proteins and DNA. According to Rao et al. (1998), a value of 35 mg/day would be an appropriate average daily intake of this antioxidant for adults.

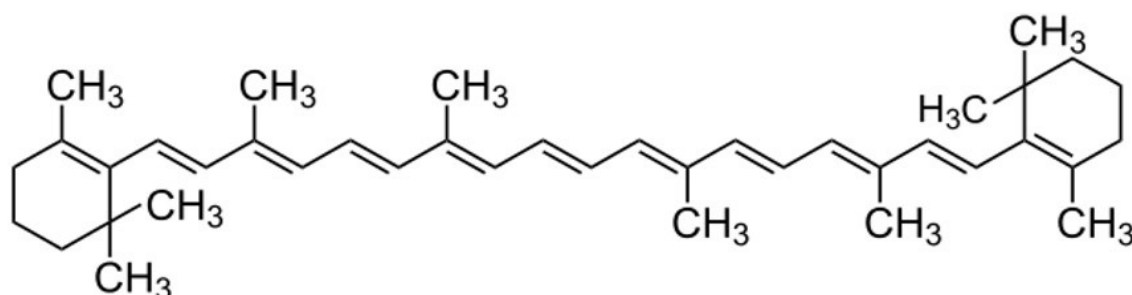


Figure 2. The basic structure of a carotenoid.

Source: Costa (2024).

Betalains (Figure 3) occur in 13 plant families of the *Caryophyllales* and have never been found to occur concomitantly with anthocyanins in the same plant. They can be found in opuntia flowers (from the *Cactaceae* family), beets, chard, pink dragon fruit, red, pink and orange bougainvillea, as well as red amaranth flowers.

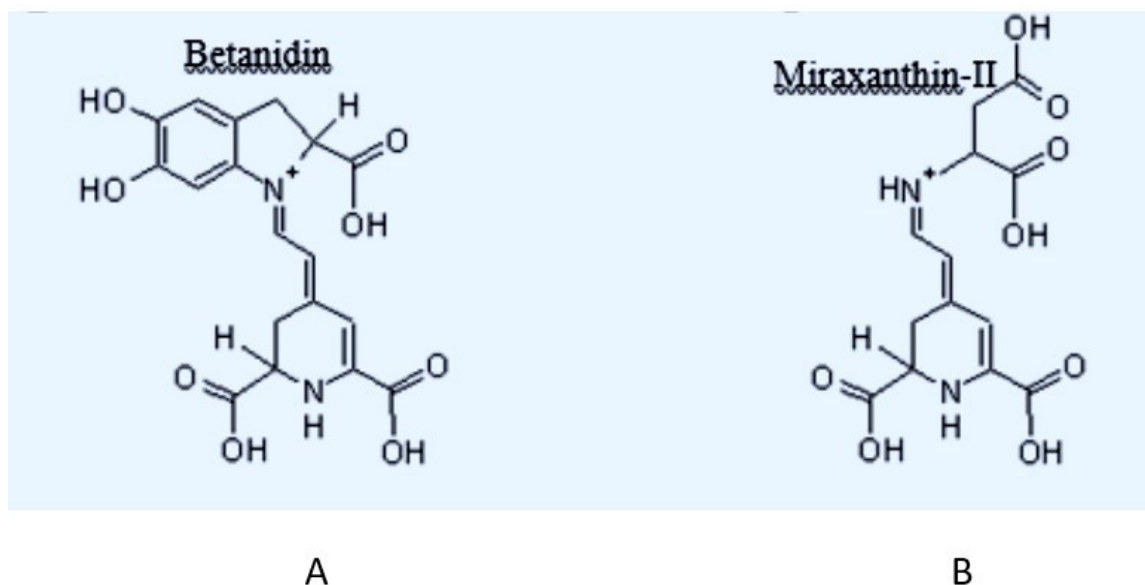


Figure 3. Betalain and its resonance structures. (A) example of betacyanin and (B) of betaxanthin.

Source: Mohamad et al. (2019).

Natural colorings exert biological activities with health benefits, including antioxidant properties, protection against oxidative damage to cellular components, anti-inflammatory activity, and prevention of chronic non-communicable diseases. Most of the evidence of benefits, however, has been verified *in vitro*, thus studies are required to confirm the physiological and pharmacological effects of natural colorings on the human body. The factors that influence bioavailability, absorption, and distribution in tissues and organs should be studied, as well as structural transformations, pH changes in the formation and degradation of products, among others.

Restrictions on the use of certain synthetic colors have greatly reduced the palette of colors available to the food industry, with the greatest impact on the red tone. The Food and Drug Administration (FDA) has banned FD&C Red 2 (amaranth) in the USA, scarlet GN and ponceau GR in France, and orange RN in the UK, which has led to an increase in demand for natural sources of red pigments (Cisse et al., 2009). Natural reds used successfully in food include betanin, cochineal, carotenoids, and mainly anthocyanins. It is worth mentioning that the use of natural colorants requires detailed knowledge of the processing and storage conditions that can affect the stability and lead to degradation of the pigments (Moura et al., 2019a).

The search for healthier products requires the industry to make available natural colorings that can be used in food products, with a wide pH range and that are stable to thermal processing and during storage in ambient, refrigerated and frozen conditions (Nachay, 2017a). Encapsulation is an alternative to improve the stability and bioavailability of natural colorings (Silveira et al., 2023). The use of natural colorings in the form of extracts containing encapsulated polyphenols, rather than free compounds, can overcome the disadvantages of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound *in vivo* and *in vitro* (Budin et al., 2023; Silveira et al., 2023).

Several strategies have been developed to protect polyphenols from adverse environmental conditions, which include the following: (1) changing its structure by chemical structural modification (glycosylation, acylation); (2) combining them with biological macromolecules such as proteins and polysaccharides to form stable complexes; (3) micro-/nano-encapsulation to provide a physical barrier that shields the anthocyanins from environmental factors; and (4) co-pigmentation. Moreover, these strategies help minimize degradation and preserve the structural integrity and functional properties of anthocyanins over extended periods, enhancing their stability during storage and processing. However, some encapsulation methods are not widely used in food industries due to their high production cost (Saini et al., 2024).

2.7 Natural flavorings

Recently, new aroma extraction techniques have been used to replace conventional techniques (steam distillation or the use of solvents) to reduce environmental impact, energy consumption, and processing time, as well as avoiding the use of organic solvents and improving environmental sustainability by using agro-industrial waste and adding value to co-products (Tylewicz et al., 2017). Although conventional extraction techniques using heat treatment provide safety for aromatic ingredients, they accelerate chemical reactions (Maillard reaction, caramelization, lipid oxidation) and lead to losses of aromatic compounds. Thus, emerging technologies such as supercritical fluid extraction, ultrasound, microwave or enzyme-assisted extraction, ohmic-assisted hydrodistillation (OAHD), high pressure, and nanotechnology have been studied and used by some industries. A practical example is the use of flavoring compounds such as essential oils (EOs) extracted from leaves, fruits and seeds and preserved through nanoencapsulation technology (Nair et al., 2022). Another example is innovative strategies for biocatalytic flavor generation taking advantage of the immense progress currently being made in the emerging fields of functional genomics, proteomics, protein engineering and metabolic engineering (Schrader et al., 2004).

The safety assessment of aromatic ingredients for classification as a Generally Recognized As Safe (GRAS) ingredient must be rigorous. Several factors are considered in this assessment, including the history of use as a food, forms of use, daily intake, extraction method, the chemical composition of essential oils and natural flavoring extracts, and safety assessment. This last item considers exposure, the potential toxicity of the identified constituents, the genotoxicity of unidentified constituents, and global safety, considering the safety of consumption by children and individuals with low body weight (Gooderham et al., 2021).

Natural flavorings include extracts from different plants, such as fruits, coffees, teas, herbs, spices, and other botanicals. The use of different extraction technologies allows for the production of different forms of presentation (liquids and powders), and preservation of the natural aroma. Yeast extract is a flavoring agent widely used because it is natural and contains aromatic notes suitable for use in vegan products and meat

analogs. This extract is made up of non-volatile substances, consisting of peptides, nucleotides, B vitamins, and amino acids (Liu et al., 2008 2015), in addition to the volatiles and aromatic compounds generated during the heating of these substances. Sourdough can also contribute to the formation of distinctive aromas during baking, which leads to the hydrolysis of proteins with the release of free amino acids, and the formation of organic acids, alcohols, and esters when used during the prolonged fermentation of bakery products. Flavors from sourdough can vary depending on the fermentation conditions (time, temperature, pH), product composition, and the type of starter culture used during baking (Hansen & Schieberle, 2005).

Flavor is characterized as the mixture of aroma and taste perceptions. Flavor can also be classified based on the parameters of mouthfeel and flavor richness. Aroma compounds are very volatile molecules and are mainly perceived through the nose. Taste receptors are in the mouth and, when food is chewed, flavors are perceived.

Flavorings are used in the processed food and beverage industries. These additives strengthen taste properties and mask undesirable flavors and aromas. Consumers seem to prefer products that do not contain artificial ingredients and are willing to pay high prices for them. Several authors point out that artificial flavors are harmful and contribute to the majority of cancer and health conditions worldwide. The flavors are characterized by the sensorial analysis of the material. Natural flavorings usually come from essential oils. Artificial flavorings, on the other hand, are made from various compounds that react and form a specific concentrated flavor (Mosia et al., 2022).

All flavors, including those intrinsically present in foods, as well as natural and artificial flavoring additives, are small chemical compounds or mixtures of compounds. The well-defined structures of these compounds allow them to interact with each other during the chewing process and bind to taste receptors in our mouth, thus creating distinct flavors.

Artificial sweet flavorings have a complex composition consisting of several classes of chemical compounds that are not specified or not permitted by regulatory bodies and industries responsible for their production and the amount of chemical formulation that may cause adverse effects some of examples are I. Cytotoxic and genotoxic potential II. Hyperactivity, anxiety and depression effects (Singh & Sudha, 2024)

The term artificial flavorings means any substance whose function is to impart aroma, which is not derived from spices, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or plant material similar, meat, fish, poultry, eggs, dairy products or fermentation products thereof.

Most of the natural flavoring compounds are produced from the oils of plant products. These oils contain alkaloids which dissolve in organic solvents like alcohols. The flavorings that are extracted from fruits and spices are often synthesized using special processes and are manufactured in such a way that they remain unchanged in their aroma and flavorings at the end of the extraction process (Singh & Sudha, 2024). Table 1 presents examples of artificial and natural flavorings.

Table 1. Artificial flavoring agents, their applications in the bakery industry and the type of flavors they induce. Natural flavors and their respective substrates.

Artificial flavor	Confectionery product	Flavor induced
Diacetyl	Buttercream, icing, croissant	Intensely buttery and warm flavor
Ethyl decadienoate	Jam fillings and pastries	Apple, pear and grape-like
Ethyl vanillin	Cakes, ice creams, cookies, truffles, biscuits, buns, croissants and chocolates	Strong vanilla or chocolate-like
Synthetic limonene	Marmalades, candies, lemon tarts, pastries and cake frosting	Citrus fruits-orange, lemon and sweet lime
Natural flavor	Substrate	
Isoamyl butyrate	Essential oil isoamyl alcohol + butyric acid	--
Flowery and fruity smell	Acetate like ester compounds	--
Vanillin	Eugenol via ferulic acid (<i>Pseudomonas</i> sp.)	--
Intense aroma of cooked fruits	Wheat bran + cassava bagasse + sugar cane bagasse + glucose	--

According to Singh & Sudha (2024), natural flavorings can also be said as artificial flavorings because they are not created only from the food but are replicating and mixed with other products to create a flavor. Whereas artificial flavorings are made solely from chemicals combined to mimic a flavor. FDA does not require food labels to say what is in their “natural flavorings” unless the ingredients include a common allergen like milk, egg, fish, shellfish, tree nuts, wheat, peanuts or soy.

2.8 Vegetable sources for nitrite alternatives

Meat and poultry curing is one of the oldest forms of food preservation still in use today. Before the advent of refrigeration, fish and meat were preserved by methods found effective in controlling spoilage after animal harvest and extending food supplies during times of scarcity. Although lost in antiquity, the curing process for meats is believed to have derived from preservation methods with salt as early as 3,000 B.C. (Romans et al., 2001). Meat curing originated due to the generation of pink color in salted meat. Subsequently, potassium nitrate contamination in salt was identified as the causative agent of meat curing (Honikel, 2008). Over time, the realization that salt contaminated with saltpeter (potassium nitrate) was responsible for curing, would unknowingly provide the basis for the beginnings of unraveling the mystery of curing (Honikel, 2008; Sindelar & Milkowski, 2012).

Currently, meat curing is used to improve the quality, safety, and shelf life of meat products using various ingredients and additives such as salt, nitrite, nitrate, phosphate, etc. (Sebranek, 2009). Nitrite is an essential component of the additives that are used for meat curing and imparts a unique pink color and flavor to the meat (Parthasarathy & Bryan, 2012). Also, nitrite acts as an antioxidant against lipid oxidation and inhibits the growth of spoilage and pathogenic bacteria including *Clostridium botulinum* and *Listeria monocytogenes* (Sebranek, 2009). Synthetic nitrite, such as sodium nitrite and potassium nitrite, is commonly used in the meat industry. Nevertheless, consumers' concerns about synthetic additives and their preferences for the consumption of natural and organic food have considerably increased in recent times. Therefore, various studies have been conducted to replace synthetic additives with natural products (Flores&Toldrá, 2021; Shakil et al., 2022; Jo et al., 2020).

Color of cured meat is one of the most noticeable effects of nitrite in meat products. Meat color is highly variable and is influenced by a variety of factors. When nitrite is added to meat, it is converted to nitric oxide (NO) via the reactions listed below (Skibsted, 2011; Shakil et al., 2022):



Nitrite reacts with hydrogen ions (H^+) of water to produce nitrous acid. After that, nitrous acid progressively decomposes into water molecules (H_2O) and di-nitrogen trioxide (Equations 1 and 2). Then, nitric oxide and nitrogen dioxide are generated from dinitrogen trioxide (N_2O_3) (Equation 3). The major component responsible for nitrite's apparent function in cured meat products is nitric oxide (Shakil et al., 2022).

Nitric oxide combines with the iron of both myoglobin (Fe^{2+}) and metmyoglobin (Fe^{3+}) to produce a cured pink color in meat (Skibsted, 2011). Myoglobin is the sarcoplasmic protein responsible for the red color in meat, and metmyoglobin (brown in color) is the oxidized form of myoglobin (Mb). When nitric oxide (NO) reacts with myoglobin (Fe^{2+}) nitrosyl myoglobin is formed. The bright red nitrosyl-myoglobin complex is responsible for the distinct color of cured meat. This complex is extremely unstable, and it turns into a stable, brilliant reddish-pink pigment (nitroso-hemochrome) during heat treatment (Sebranek & Fox Junior, 1985).

Furthermore, myoglobin may react with HNO_2 . Myoglobin (Fe^{2+}) combines with nitrous acid and forms metmyoglobin (Fe^{3+}) by oxidation. Metmyoglobin (Fe^{3+}) then reacts with NO to produce NO-metmyoglobin. NO-metmyoglobin is also produced from the reduction of metmyoglobin. As a result, the meat becomes brown in color. NO-metmyoglobin can be converted to NO-myoglobin by a reductant, causing the formation of the cured color (pink) again when heated. The presence of other additives in cured meats also affects the color development. Antioxidants including erythorbate, ascorbic acid and polyphenols stimulate the production of NO by allowing the N_2O_3 reduction. Ascorbic acid reduces Fe^{3+} to Fe^{2+} effectively and enhances the reduction process of NO-metmyoglobin. Thus, antioxidants with reducing activity aid in the cured meat color development by raising NO production and lowering NO-metmyoglobin levels. NaCl, generally added to meat for curing, reacts with HNO_2 to generate nitrosyl chloride, which is more sensitive than N_2O_3 in terms of generating nitric oxide (NO) and initiating the formation of NO-myoglobin. The rate of nitrosyl myoglobin production has been found to increase with increased salt concentration (Sebranek & Fox Junior, 1985). The pH also controls nitric oxide formation from nitrite. Nitrous acid (HNO_2) and nitrite reactivity increase as pH decreases. A very small quantity of nitrite is required for the development of the cured color in meats, usually approximately 2-14 ppm (Tarté, 2009). The recommended residual nitrite to function as a reservoir for the cured meat color regeneration is usually, 10-15 ppm (Honikel, 2008).

The meat and poultry industry has greatly benefited from the use of sodium nitrite by allowing for the production of products with unique colors, textures, and flavors; improved food safety; and an extended shelf life with excellent storage stability. The use of sodium nitrite for curing, however, has not been without controversy. In 1970, Lijinsky & Epstein (1970) published a critical report in *Nature* entitled “Nitrosamines as Environmental Carcinogens”, which showed that nitrosamines were potent and specific carcinogenic compounds. Further, the authors concluded the most appropriate means to address the problem was to eliminate one or the other of nitrosamine precursors (nitrite and secondary amines). This particular paper brought widespread public attention to the question of the safety of nitrite and was followed by an intense survey and study of potential public health risks due to food and environmental exposure to nitrite. Since all cured meats were viewed as containing both precursors, consumption of cured meat was considered a potential public health hazard. Due to a strong public debate in the 1970s concerning the potential to yield carcinogenic nitrosamines, the use of nitrite for curing was nearly banned (IARC, 2010). Since then, several steps have been taken by both industry and government to significantly reduce the risk of nitrosamine formation and alleviate potential human health concerns. Since that time, health concerns involving risks related to cancer believed to be directly related to the consumption of nitrite-cured meat and poultry products, have periodically resurfaced (Sindelar & Milkowski, 2012). However, research conducted since the mid-1980s has suggested that nitrite is a significant molecule important for human health. New scientific discoveries are now providing a better understanding of the important role nitrite plays in human physiology. Dietary nitrate from vegetable consumption, for example, has been shown to serve as a significant source for the endogenous production of nitrite and nitric oxide in the human body (Bryan & Ivy, 2015).

Although it is generally recognized that nitrite influences the meat flavor, the reactions involved are not completely understood. The antioxidant activity of nitrite against lipid oxidation is assumed to be one of the methods which might alter the flavor of meat products by suppressing the “warmed-over” flavor. Aldehydes such as pentanal, hexanal, etc., which are the products of lipid oxidation, are suppressed in cured meat when lipid oxidation is inhibited by nitrite. The use of nitrite inhibits the formation of aldehydes (hexanal), masking the sulfur-containing chemicals that give cured meat its flavor (Shahidi, 2002). S-nitroso thiol production and disulfide bond breakdown during meat curing are likely to cause increases in sulfur compounds. The antioxidant effect of nitrite explains why oxidation products, such as hexanal, are reduced in cured meats. Also, a nitrite may prevent rancidity during storage and the formation of “warmed-over” flavors. More research is needed to completely understand the mechanism, reactions and volatile compounds responsible for the aroma and flavor of cured meat (Shahidi, 2002).

For cured meat products, nitrite is recognized for its antimicrobial effects against pathogenic bacteria, even though the specific inhibitory mechanisms are not well known. Nitrite contributes to oxidative stress by being

the precursor of peroxynitrite (ONOO^-), which is the major strong oxidant. Thus, bacterial stress is enhanced by the nitrate-nitrite-peroxynitrite system which is also highly pH- and low partial pressure of oxygen-dependent. Nitrite is a hurdle technology whose effectiveness depends on several other hurdle technologies including sodium chloride (accelerating the autoxidation of oxymyoglobin and promoting peroxynitrite formation), ascorbate (increasing ONOO^- synthesis), and water activity (A_w). Depending on the environment, certain species are more resistant than others to acidic, oxidative, and nitrite bacteriostatic effects (Shakil et al., 2022). The most resistant species are gram-negative aerobic/facultative anaerobic bacteria (*Escherichia coli*, *Salmonella*), and the most fragile are gram-positive anaerobic bacteria (*Clostridium botulinum*). There are two effects of nitrite found in controlling the growth of *Cl. botulinum*. The first effect is inhibiting vegetative cells developing from surviving spores. The second effect is the prevention of vegetative cell division. In addition, numerous studies have found that nitrite inhibits the development of *Listeria monocytogenes*, *Bacillus cereus*, *Cl. perfringens* in various meat products. The impact of nitrite and inhibitory mechanisms varies with several bacterial species. The effectiveness of antimicrobial activity is dependent on various factors including pH, residual nitrite level, salt concentration, Fe content, reductant presence, and storage temperature. The antibacterial activity of nitrite may be due to the peroxynitrite (ONOO^-) formation and nitric oxide formation from nitrite (Majou & Christeans, 2018). Acid catalysis may cause oxymyoglobin to be autoxidized, generating superoxide radicals. The interaction of nitric oxide with superoxide radicals as well as the reaction of nitrite with hydrogen peroxide can produce peroxynitrite. Under physiological environments, peroxynitrite and peroxynitrous acid (ONOOH) stay in equilibrium. These two compounds are strong oxidants as well as nitrating agents. They penetrate the bacterial cells by passive anionic diffusion and disrupt the microorganisms by causing protein and lipid oxidation or by damaging DNA. Nitric oxide (NO) can also inhibit microbial growth by forming protein-bound dinitrosyl iron complexes (DNICs) when it reacts with iron-sulfur proteins, which are engaged in critical physiological activities including energy metabolism and DNA synthesis. Various kinds of microorganisms have various metabolic pathways and antioxidant defense strategies, and certain microorganisms are found to be resistant to the oxidative stress of peroxynitrite and peroxynitrous acid. Furthermore, the antibacterial action of nitrite in Gram-positive anaerobic bacteria has been shown to be more effective than in Gram-negative aerobic bacteria. The level of added nitrite is thought to have a greater influence on inhibiting *Cl. botulinum* than that of the residual nitrite during storage, implying that the production of antimicrobial compounds as a consequence of nitrite-related reactions might be noteworthy (Majou & Christeans, 2018).

Sodium nitrite is essential in the production of meat products. Its preservative effect and its contribution to the development of the characteristic taste and pink color of cured products are widely known, as well as its ability to delay oxidation and the appearance of undesirable aromas. However, despite all the technological benefits of nitrite at the safe levels allowed (150 ppm in most products), there is pressure from the market to reduce or exclude this ingredient from meat products since many consumers perceive nitrite-free products as more natural and healthier (Tarté, 2009).

The alternative available to replace synthetic nitrite is the addition of a nitrate source that is converted into nitrite by the action of reductase enzymes present in microorganisms such as *Staphylococcus carnosus*, which is very common in starter cultures used to make fermented products. Dehydrated concentrated vegetables (celery, beet, spinach, and carrots, among others) and sea salt can be used as a source of nitrate. In this case, the label should state “No nitrates added or nitrites except naturally occurring nitrates” (Flores & Toldrá, 2021).

Antimicrobial compounds of natural origin can also be used to replace or complement the effect of nitrite in meat products. These compounds are naturally present in certain spices that contain essential oils rich in terpenes, coumarins, and flavonoids. There are also compounds of microbial origin (nisin, for example) or animal origin (lysozyme and some polypeptides). The total replacement of nitrite with a single antimicrobial agent of natural origin is not always possible, thus the addition of combinations of different compounds has proven to be more effective (Rivera et al., 2019).

The first studies on the use of fermented plant compounds as a source of nitrate were carried out to meet the needs of the organic product segment, which needed to produce cured products with no addition of synthetic preservatives (Sebranek et al., 2012). The use of nitrate-rich plant extracts together with starter cultures of bacteria-producing nitrate-reducing enzymes has been proposed, emphasizing the need to control the nitrite content formed to levels that guarantee the microbiological safety of the product (Sindelar, 2006; Sebranek&Bacus, 2007). The use of acerola extract as a source of ascorbic acid to replace sodium erythorbate as a cure accelerator has also been recommended, as this extract reduces the residual nitrite content without affecting the pH, thus providing an alternative curing process (Sullivan et al., 2012a).

The conversion of nitrate into nitrite during the processing of the meat product requires adjustments that include changes in pH and the incubation time, among other parameters. The bacteria that make up the starter cultures require incubation between 38 and 42 °C for around two hours to promote the reduction of nitrate, which requires adjustments to both the formulation and heat treatment, especially in small-caliber products (e.g., sausages), which are subjected to a rapid thermal process. This alternative curing process with the addition of concentrated vegetable juices from nitrate-rich plants has been used by different industries at the beginning of this century, mainly in the United States of America (USA) (Sullivan et al., 2012b; Ko et al., 2017). To better control alternative curing, these concentrates were fermented before their addition to products, which also created many inconveniences for the meat industry. Therefore, flavoring suppliers produced dehydrated fermented plant concentrates, a source of pre-converted nitrite, to replace synthetic nitrite, with no significant process modifications. This technological evolution has led to an increase in the supply of alternative cured meat products, as can be seen in some markets mainly in the USA and Canada. In these countries, manufacturing volumes have been growing since the mid-2000s to meet the consumers' demand for products free from chemical additives. The first fermented or non-fermented plant concentrates were made with celery; today different sources are used in commercial products, such as beet, spinach, and recently chard, which, unlike celery, is not classified as an allergen (Flores & Toldrá, 2021).

Recently, many studies have focused on the production of different meat products using an alternative curing method with plant extracts (Yong et al., 2021; Flores & Toldrá, 2021). In the search for other synthetic coadjuvant compounds in the curing process, various ingredients of natural origin have been evaluated, such as concentrated lemon juice and acerola powder (sources of vitamin C, ascorbic acid) to replace cure accelerators such as sodium erythorbate, as well as the use of other natural bacteriostatic agents, such as fermented sugar and buffered vinegar. These implementations aim to intensify the microbiological safety of this category of products, which is achieved with compounds such as sodium lactate and diacetate, among others (Golden et al., 2017; Rasmussen, 2018).

In meat products, the plant substitutes for synthetic nitrite provide a similar amount of nitrite, thus the microbiological safety of the product is not affected (Rasmussen, 2018). On the other hand, depending on the product category, it is not always possible to achieve the same concentration of added nitrite. In such cases, the formulation must be altered to include other antimicrobial compounds that have a synergistic effect with nitrite, so that the product remains microbiologically safe for consumption. These antimicrobial agents include buffered vinegar and fermented sugar (King et al., 2015; Golden et al., 2017; Sullivan et al., 2012a).

It is known that the addition of 90 ppm nitrite prevents the germination of *Cl. botulinum*, a sporulated microorganism whose toxin can be lethal for most meat products except for long-cured meats (Tarté, 2009). On the other hand, there are other pathogens of public health importance, such as *Cl. perfringens*, *Listeria monocytogenes*, *S.aureus*, and others. Thus, *Cl. perfringens*, in particular, is very relevant in ready-to-eat baked goods sold under refrigeration, once other barriers such as *A_w* and pH are not sufficient to guarantee safety. *Cl. perfringens* grows in the temperature range between 12 and 50 °C and has a high multiplication capacity in the range of 45 to 47 °C, thus preventive measures must be established from the time of cooling after heat treatment, especially when the product has a large caliber (McMinn et al., 2018). Different studies using synthetic nitrite have shown that the addition of at least 100 ppm of nitrite is sufficient to prevent the

germination of *Cl. perfringens* spores (Fraqueza et al., 2020; Gipe, 2012), which is possible when using concentrated fermented plant extracts (pre-converted nitrite). In turn, initial levels are generally lower when using nitrate-rich plant concentrate with a culture of nitrate-reducing bacteria (e.g., *S. xylosus*), leading to a need to use bacteriostatic agents (Jackson, 2010; King et al., 2015). Studies have also shown that the effects on preventing *Clostridium* germination are similar whether the cure accelerator is synthetic or of natural origin (acerola powder) when used in equivalent concentrations (Gipe, 2012; Sebranek et al., 2012).

The nitrite concentration of the nitrate-rich plant concentrate should be considered when using it as a nitrite source, as the technical data sheet usually provides information as nitrite and/or sodium nitrite, informing the equivalent content based on molecular weight for comparison purposes. The nitrite concentration varies according to the type of plant and the production process. Concerning the cure accelerator, traditional products use sodium erythorbate, while a plant extract rich in ascorbic acid is often chosen in the category of clean-label products (King et al., 2015). The concentration of ascorbic acid in these ingredients (e.g., acerola powder) is variable and calculations must be made to ensure that the content added corresponds to the synthetic product, considering that ascorbic acid promotes faster nitrite depletion when compared to sodium erythorbate when used at the same concentrations.

Concerning the legislative aspects, the USA was the first country to regulate this category of meat products made with plant concentrates as alternative curing agents (United States Department of Agriculture, 2018). These ingredients are considered natural once the manufacturing processes (concentration and drying or fermentation) meet the regulatory requirements. To be labeled as natural, all the ingredients in the formulation must be of natural origin. It is worth noting that Brazil does not have a regulated definition for the use of the term “natural” in food labeling. On the other hand, US legislation authorizes the term “preservative-free” when the product has a plant concentrate added, even if it is an additive. The use of the terms “uncured” and “with no nitrite and nitrate addition” is permitted, despite being controversial because the product contains nitrite. This nomenclature should be revised at the request of consumer associations and academic representatives, since the products still contain nitrite, which is the active compound in the curing process, even if it comes from a plant source. The legislation also establishes criteria for marketing this category of product, as well as cooling requirements. Products cured with plant extracts must be stored at temperatures of up to 4 °C unless they are frozen or sterilized. The addition of nitrite is not necessary for products with a maximum Aw of 0.92 (Fraqueza et al., 2020).

Although the USA and Canada have allowed the use of plant extracts as a source or precursor of nitrite for many years and classify these ingredients as flavoring agents, their use is not permitted in Europe, as they are considered to have a technological function as preservatives and should therefore meet the relevant regulations, including purity criteria (Flores & Toldrá, 2021).

Numerous studies have been carried out since 2010 to assess the safety of products made with the replacement of synthetic nitrite with nitrite from natural sources, especially concerning the pathogens *Cl. botulinum*, *Cl. Perfringens*, and *L. monocytogens* (Flores & Toldrá, 2021).

Pathogenic microorganisms are isolated from meat products, however, they may not represent a danger, once the products do not always meet all requirements for bacterial growth. In this case, challenge tests with the product can be very effective. For that, pathogens are inoculated under process and marketing conditions similar to real conditions, and their growth potential and toxin formation are assessed. When bacterial growth is detected in the product, protective barriers established by intrinsic and extrinsic factors must be adopted to guarantee the microbiological safety of the product. However, in practice, there is not always evidence of growth and/or toxin production (National Advisory Committee on Microbiological Criteria for Foods, 2010).

Jackson (2010) evaluated the microbiological safety of sausages, cooked ham, and bacon made with synthetic nitrite substitution by inoculating *Cl. perfringens* in commercial products and observed a wide variation in growth between brands. The author also evaluated different bacteriostatic compounds in sausages and cooked ham and concluded that vinegar and fermented sugar prevented growth in products cured with

plant extracts. Regarding the germination and growth of *Cl. botulinum* in sausages and cooked ham, the products showed no growth of the pathogen at the storage temperatures evaluated (22 °C, 10 °C, and 4 °C) when fermented sugar and vinegar were added to products made with pre-converted nitrite source (concentrated fermented celery juice). In turn, the bacteriostats lemon, acerola, and vinegar only prevented growth at temperatures of 4 °C and 10 °C, while the pre-converted nitrite source, without the addition of a bacteriostatic agent, only prevented growth when the product was stored at 4 °C.

The use of natural alternatives to nitrate and nitrite or natural sources of nitrite and nitrate such as vegetable extracts may have relevant benefits since it allows the launch of clean label meat products. However, the risk for the generation of N-nitrosamines remains because some nitrite is present in the meat product. Anyway, such risk is low if the amount of added nitrite is controlled and remains very low in the meat product. Other risks might be associated with the presence of other contaminants such as mycotoxins, heavy metals, organic pollutants that may be present in the vegetables used as sources and the allergenic potential from the vegetable (i.e. celery) (Flores & Toldrá, 2021).

The use of plant concentrates or extracts as nitrate sources has not yet been regulated in Brazil, which should be evaluated by Agência Nacional de Vigilância Sanitária (ANVISA) to define the regulatory guidelines.

The safety of meat products depends on several factors, including pH, A_w , preservatives, type of packaging (redox potential), and mainly storage temperature. These different factors act synergistically and the greatest impact depends on the product category, composition, and form of marketing, among other characteristics.

2.9 Challenges of replacing sodium, sugar, and fat in food

The global prevalence of diabetes can reach 700 million people by 2045. According to the International Diabetes Federation (IDF), 79% of diabetics live in developing countries, and 63% of this total is of working age (up to 60 years) (International Diabetes Federation, 2019). Obesity has been identified as a risk factor for diabetes and several other diseases and has been associated with an inadequate diet, especially the consumption of salt, sugar, and fat, among other factors.

Reducing the salt content and replacing all or part of the sugar and fat in food is a challenge for manufacturers, due to their important technological, sensory, and preservation properties. These ingredients are determining factors not only in enhancing flavor and texture but also in controlling microbial growth. Sugar also plays a fundamental role in the texture of some products, as a body and viscosity agent (Costa et al., 2021). In the search for substitutes for these ingredients, it is necessary to consider consumer preferences for certain sensory properties, as well as ensure the quality and safety of the food during storage (Guerra et al., 2021).

Technologies using AI to develop digital devices in the area of personalized nutrition have emerged on the market. This technology uses biomarkers that, by tracking the user's metabolic health, help them manage their health and propose lifestyle changes that take into account individual specificities (Nutrition Insight, 2021). This market is expected to grow in the coming years, especially due to the rise in obesity-related chronic non-communicable diseases (NCDs), such as diabetes.

2.10 Sodium reduction

Sodium is an essential nutrient necessary for the maintenance of plasma volume, acid-base balance, transmission of nerve impulses, normal cell function and regulating physiological functions (World Health Organization, 2023; Nie et al., 2024). However, excess dietary sodium has been associated with some chronic non-communicable diseases. According to the World Health Organization (2023), a maximum daily intake of 2,000 mg of sodium is recommended, which corresponds to around 5 g of sodium chloride or table salt.

Reducing sodium chloride in food can influence different aspects of food processing, quality, and preservation. In bakery products, the reduction affects the rheology of wheat flour dough, with a consequent impact on the technological and sensory quality, shelf life, and microbiological safety of the products (Silow et al., 2016).

In wheat flour pasta, NaCl promotes a more stable, less extensible, and less sticky gluten network. In addition, sodium ions affect the yeast cell wall, controlling fermentation and reducing gas production, thus reducing NaCl can result in breads with a more fragile texture and more open crumb structure, as well as affecting Aw and decreasing product stability (Cauvain, 2007; Silow et al., 2016).

The reformulation of bakery and meat products involves different approaches, including the replacement of sodium chloride by different salts or its mixture, such as potassium chloride, magnesium chloride, calcium chloride, magnesium sulfate, potassium lactate, calcium lactate, potassium phosphate, and others (Kaur et al., 2011; Israr et al., 2016; Nie et al., 2024), or modification of the salt structure (particle size reduction, encapsulation, porosity and morphology) (Silow et al., 2016; Nie et al., 2024); addition of flavor enhancers, such as amino acids (monosodium glutamate and L-alanine), nucleotides (disodium inosinate and disodium guanylate), organic acids (disodium succinate), plant compounds (allicin and gingerol) and peptides that increase salinity (Maillard peptides and extracts of yeast) (Nakagawa et al., 2014; Nie et al., 2024).

The halophyte plants, which due to their health benefits and salty flavor have been presented as an option to sodium chloride (*Sarcocornia spp.* and *Salicornia spp.*) (Louçano et al., 2024).

In meat products, sodium chloride also plays an important role, conferring salty taste and improving the flavor perception of other ingredients. It also acts as a preservative, and promotes the solubilization of myofibrillar proteins, with an effect on the water retention capacity and emulsification.

The most common way of reducing sodium in meat products is to replace up to 50% of NaCl with KCl, combined with the addition of flavor enhancers to mask the bitter taste of KCl. Another alternative is to reduce the particle size of the salt, which increases the perception of the salty taste and allows for a reduction in the salt content without impacting the sensory characteristics of the product.

The main barriers to be faced in the manufacture of low-sodium meat products include texture, process yield, exudation during storage, and microbiological stability. The antimicrobial activity of salt is largely related to its effect in reducing Aw. Salt is part of a multiple barrier system for food preservation; therefore, salt reduction requires adjusting the intrinsic or extrinsic properties of the food to guarantee effective preservation. Any reformulation requires an investigation of the hazards arising from the changes and appropriate action must be taken in the face of new hazards (Stringer & Pin, 2005).

The addition of other compounds to compensate for the loss of functionality associated with salt reduction must be monitored against associated changes in the microbiological flora. Although reducing salt concentrations generally does not alter the number or species of bacteria initially present, it can affect their survival and growth. The magnitude of an increase in pathogen growth associated with salt reduction depends on the contribution of salt to the safety of that product. For many food groups, such as frozen, sterilized, acidic (pH < 3.8), and low Aw foods (<0.86) there is no implication of salt reduction in microbiological safety.

One of the main strategies to reduce sodium is the replacement of sodium chloride with other salts, especially KCl, as mentioned above. However, according to Nie et al. (2024), additional randomized clinical trials should be carried out to better understand the effect of potassium in chronic kidney disease patients and normal populations, as well as its independent effects on cardiovascular diseases.

However, replacing sodium with all properties, whether technological, sensorial, microbial stability, as well as physiological effects, is a challenge for the food industry, and often requires the combination of substitutes due to the complexity of the food system and its processing.

2.11 Sugar reduction

Currently, there is a wide variety of ingredients used as sucrose substitutes. Table 2 shows the intensive sweeteners responsible for giving a sweet taste. This category includes aspartame, acesulfame-K, sucralose, saccharin, cyclamate, stevia, neotame, thaumatin, monk fruit (luo han guo), and others (Barreiros, 2012). According to Table 2, the advantage stands out, which has an Acceptable Daily Intake (ADI) of 500 mg/kg and a high energy value, close to that of glucose (3.8 kcal/g), however, the amount needed to sweeten is small due to its sweetening power be high (20,000 to 37,000 times). As for natural products, thaumatococcus stands out, which has a greater sweetening power (1,300 to 3,500 times), and zero kcal/g, however, its ADI has not yet been established. Regarding the use of stevia, it should be noted that this natural sweetener has a sweetening power of 300 times, but with a small ADI, of only 4 mg/kg, which somewhat limits its use, and the strategy is to combine it with other sweeteners or use high purity stevia.

Table 2. Composition, sweetening power, cariogenic potential, ADI, thermal stability, nature, and energy value of sweeteners.

Type	Composition	Sweetening power ¹	Cariogenic potential	ADI	Thermal stability	Nature	Energy value
TRADITIONAL SWEETENERS							
Sucrose	Glucose and fructose	1 (Sugar Reference)	Yes	14-60 g/day	Yes	Natural	4 kcal/
Fructose	Monosaccharide	1.7 X	Yes	50 g/day	Yes	Natural	4 kcal/
Glucose	Monosaccharide	0.7 X	Yes	50 g/day	Yes	Natural	4 kcal/
INTENSE SWEETENERS							
Saccharin	Methyl anthranilate	300 X	No	50 mg/kg children 1,000 mg/kg adults	Yes	Artificial	0 kcal/g
Cyclamate	Cyclohexyl-Sulfamic acid	30 X	No	11 mg/kg	Yes	Artificial	0 kcal/g
Aspartame	L-aspartate and L-phenylalanine	200 X	No	40 mg/kg	< 40 °C	Artificial	4 kcal/g
Acesulfame-K	Organic salt: N, O, H, S, and K	200X	No	15 mg/kg	Yes	Artificial, acetic acid derivative	0 kcal/g
Sucralose	Sucrose with replacement of 3 hydroxyl groups by 3 chlorine atoms	400 - 800 X	No	15 mg/kg	Yes	Artificial	0 kcal/g
Alitame	Aspartic acid, alanine, and starch	2.000 X	No	100 mg/kg	Yes	Artificial	4 kcal/g
Neotame	Aspartic acid, phenylalanine	7.000 - 13.000 X	No	2 mg/kg	Yes	Artificial	0 kcal/g
Thaumatococcus	Extracted from Katemfe fruit typical of West Africa	1.300 - 3.500 X	No	Not informed	Yes	Natural	0 kcal/g
Stevia	Stevioside (from <i>Stevia Rebaudiana</i>)	300 X	No	4 mg/kg	Yes	Natural	0 Kcal/g
Advantame	Aspartic acid, phenylalanine, and vanillin	20.000 - 37.000 X	No	500 mg/kg	Yes	Artificial	3.8 kcal/g

Sources: Adaptado de Barreiros, 2012; Food Ingredients, 2010; Chattopadhyay et al., 2014, Geraldo, 2014. IDA= Índice de Ingestão diária

Although each non-sugar sweetener (NSS) interacts with the same sweet-taste receptor to elicit sweet taste and likely results in the same physiological effects to some extent, they are not a homogeneous class of compounds, each NSS has a unique chemical structure, resulting in different sweetness intensities, organoleptic properties and routes of processing by the body (Agyapong et al., 2020; World Health Organization, 2023).

However, the safety of artificial sweeteners is questioned, and their role in the aetiology of various diseases is debated. In particular, their carcinogenicity has been suggested by several experimental studies, but robust epidemiological evidence is lacking (Debras et al., 2022, 2024), especially in humans, therefore it is important (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, 2015) a re-evaluation by public health authorities of aspartame's role in cancer development (Landrigan Landrigan, Straif, 2021; Debras et al., 2022). In the guidance report prepared by the World Health Organization (2023), the long-term NSS use was associated with increased risk of type 2 diabetes, cardiovascular diseases (CVDs) and mortality in prospective cohort studies conducted in adults. However, there is no clear consensus on whether NSS are effective for long-term weight control or if they are linked to other long-term health effects at habitual intakes within the ADI, besides significant effects were not observed or inconsistent concerning preterm births, asthma, excessive gestational weight gain, infants with allergies, etc.

According to Miao et al. (2022), there was scarce information about the structure of sweet taste receptors, thus there was an insufficient understanding of the mechanism and a paucity of methods to theoretically evaluate sweeteners receptor interactions. These authors evaluated 28 natural and artificial sweeteners by molecular docking. The contribution of hydrophobic interactions was highlighted in artificial high-intensity sweeteners. Eight representative sweetener – T1R2 – membrane systems were constructed to investigate the mechanisms of various sweetener intensities. These results provide a deeper understanding of the mechanisms of sweetener function and offer a new direction for the design of sweeteners.

Xylitol and other polyols, on the other hand, in high doses can cause gastrointestinal discomfort, including irritable bowel syndrome, flatulence, and diarrhea. Arabitol is associated with ribose-5-phosphate isomerase deficiency and Alzheimer's disease. Lactitol can cause diarrhea, cramps, and flatulence in some individuals (Paiva et al., 2020).

According to the European Food Safety Authority (European Union, 2010), Regulation (EU) No. 257/2010, regarding the program for the re-evaluation of food additives, it could be noted they have already been permitted in the European Union before 20 January 2009. This regulation also provides that food additives are reevaluated whenever necessary given the evolution of conditions of use and new scientific information.

About the natural sweeteners, the genetic and purification improvements have been performed. A typical example of these improvements is shown through stevia, which originally had a bitter aftertaste, which was reduced after improvement, as well as purification processes that minimized bitterness, making stevia much more palatable.

Another important aspect is the use of sweetener blends to improve sensory perception, providing greater synergy between sweeteners, improving their characteristics, enhancing and increasing their sweetness, and therefore contributing to the reduction of daily intake (ADI).

Products called 100% fruit are a natural alternative to added sugar since sugar has been replaced by concentrated juices, presenting a sweetening power similar to fructose, due to the sucrose molecules naturally present in the juices. The most common bases used for these juices are apple, pear, grape, melon, pineapple, citrus, and others. Other natural sources are Agave syrup, coconut sugar, and honey. Natural agave syrup is produced by extracting concentrated blue agave juice, which has high concentrations of fructose and inulin, and a low glycemic index. Coconut sugar is extracted from the sap of coconut palm flowers and does not undergo refinement.

It is common to find high-intensity sweeteners in conjunction with bulk sweeteners in food products to complete the sugar replacement and produce appropriate texture (Hutchinson et al., 1999; Siefarth et al., 2011). Common bulking agents or sweeteners used in food products include hydrogenated starch hydrolysates, isomalt, lactitol, maltitol, mannitol, sorbitol, xylitol, and polydextrose (Hutchinson et al., 1999).

Table 3 shows the bulking agents used as sucrose substitutes. These compounds are low in calories (< 4 cal/g) with no pronounced sweet taste, thus their function is to replace sucrose solids. Some agents also act as a source of fiber, such as polydextrose, inulin, fructooligosaccharides (FOS), and L-sugars, which aid digestion, contribute to the feeling of satiety, and have a positive impact on reducing blood sugar levels. Depending on the amount added, they can be claimed as a “source of fiber” on the label, which makes them even more attractive on the market (Abud & Silva, 2020).

Table 3. Method of production, sweetening power, application in food products and energy value of polyols.

Types	Manufacturing process	Sweetening power	Applications	Energy value
Erythritol	Glucose fermentation by <i>Moniliella pollinis</i>	0.7	Bakery products	0.2
Isomalt/Isomaltitol	Sugar alcohol by isomaltulose hydrogenation	0.45-0.65	Chocolates	2.0
Palatinité TM®	Sugar alcohol by hydrogenation of lactose	0.35-0.40	Cookies and confectionery	2.4
Lactitol	Sugar alcohol by catalytic hydrogenation of high maltose corn syrup	0.5-0.9	Cookies and confectionery	3.0
Maltitol	Sugar alcohol by hydrogenation of inverted sugar or fructose	0.5-0.72	Bakery products	1.6
Mannitol	Sugar alcohol by hydrogenation of glucose	0.6	Cookies and confectionery	2.6
Sorbitol	Xylose hydrogenation	1.0	Cookies, confectionery and beverages	3.0

Source: Adapted from Barreiros (2012); Food Ingredients Brasil (2010); Chattopadhyay et al. (2014) and Geraldo (2014).

2.12 Fat reduction

Trans fatty acids (TFA), obtained mainly through the process of partial hydrogenation oils (PHOs), was widely used in food products to increase the stability and extend the shelf life of foods (Zhou et al., 2024), and can be found in margarine, vegetable shortening, ghee, fried foods, and baked goods (crackers, biscuits and pies) etc. Trans fat can also be found naturally, in smaller quantities, in meat and dairy foods from ruminant animals (e.g. cows, sheep, goats) (World Health Organization, 2024).

However, epidemiological studies correlating the adverse effects of consuming foods containing TFA and coronary heart disease, obesity or type 2 diabetes have become more evident (Temkov & Muresan, 2021), being considered more harmful than saturated fats, as these increase LDL content, while trans fats increase LDL (low-density lipoproteins) and decrease HDL (high-density lipoproteins) (Benjamin et al., 2017).

In light of this evidence, since 2007, the WHO has recommended the elimination of trans fats from food, and in 2008 commitments to action were made, established in the Rio de Janeiro Declaration in June 2008. In 2018, the “REPLACE” action package, was carried out with guiding measures aimed at the global elimination of industrially produced trans fats by 2023 (World Health Organization, 2018). These six strategic action areas refer to the following actions (Table 4):

Table 4. REPLACE action package and its six strategic actions to ensure the rapid, complete and sustainable elimination of industrially produced trans fats acid (TFA).

ACTIONS
REview the dietary sources of industrially produced TFA and the landscape for required policy change
Promote the replacement of industrially produced TFA with healthier oils and fats
Legislate or enact regulatory actions to eliminate industrially produced TFA
Assess and monitor TFA content in the food supply and changes in TFA consumption in the population
Create awareness of the negative health impact of TFA among policy-makers, producers, suppliers and the public
Enforce compliance with policies and regulations

Source: World Health Organization (2018).

The REPLACE actions have roadmap developed by WHO for countries to help accelerate actions, offering six practical steps to promote the use and consumption of healthier fats and oils and the elimination of industrially produced trans fats, to be achieved through regulatory actions, also establishes solid monitoring and awareness systems for policymakers, producers, suppliers and the public (World Health Organization, 2024).

The WHO recommends the following two best-practice alternatives, the mandatory national limit of 2 g of industrially produced trans fat /100 g of total fat in all foods; and the mandatory national ban on the production or use of partially hydrogenated oils as an ingredient in all foods (Organização Pan-Americana da Saúde, 2021). In Brazil, these recommendations are carried out through RDC 632 of 2022 (Brasil, 2022).

The Pan American Health Organization (PAHO), which works with the countries of the Americas to improve the health and quality of life of their populations, proposed an action plan to eliminate PHOs from industrial production 2020-2025 to complete the elimination of TFAs and implementation of IP-TFA elimination policies in the Americas (Organização Pan-Americana da Saúde, 2021).

Several high-income countries have virtually eliminated industrially produced trans fats through legally imposed limits on the amount that can be contained in packaged foods. In low- and middle-income countries, where control over the use of industrially produced trans fats is often weaker, action is needed to ensure that the benefits are experienced equally across the world.

According to the World Health Organization (2020), the recommendations for PHO replacement can be carried out through the following alternatives. It should be considered that the lower the saturated fatty acid (SFA) content and the higher the Polyunsaturated Fatty Acids (PUFA) with omega-3 and omega-6 are, the healthier the replacement will be. However, according to this same document, depending on the substitute used, oxidative stability may be limiting for some food applications.

1. Stable plant oils: liquid at room temperature, e.g., naturally stable oils; trait-enhanced oils (high oleic oils), oils with antioxidants and emulsifiers;
2. Natural hardstocks: fats that are naturally high in SFA and solid at room temperature, e.g., animal fats; tropical oils and fats (palm, coconut, palm kernel);
3. Fully hydrogenated hardstocks: full hydrogenation turns oils into 100% SFA waxy fats, e.g., fully hydrogenated soy oil or other oils;
4. Fractionated oils and fats: use slow cooling to separate more solid and more liquid fat fractions, e.g., low melting (liquid) palm olein; high melting (solid) palm stearin;
5. Rearranged fats: fatty acids are reshuffled (“interesterified”) within the triglycerides, e.g., chemically or enzymatically rearranged hardstocks;
6. Blending of oils and fats, e.g., mix of soy oil and palm oil, which gives a viscous liquid;
7. Combinations of approaches 1-6, e.g., liquid oil interesterified with a specific hardstock or fractionated oil.

3 Final remarks

The search for healthy food is a growing trend, with major challenges for the clean label market, which is looking for alternative technologies for the development of new products, combined with ethical and sustainability aspects.

From the food industry approach, each type of product has a specific characteristic that must be studied before selecting a substitute. Most of the time, a combination of substitutes is indicated since there is no multifunctional clean-label substitute. Not every natural ingredient can be considered safe, just as not every artificial ingredient can be considered worse than a natural ingredient.

For the consumer, the label is a tool to manage their food choices. Individuals who are allergic, intolerant, or have diet-related diseases can access the information on the label to avoid consumption or contact (traces). The label is also a facilitator for selecting foods that are free of additives, sodium, fat, or sugar for people with chronic NCDs or those looking for a healthier diet.

Regulatory bodies and food industries play a fundamental role in the process of raising consumer awareness about the importance of their food choices. Making healthy foods available on consumers' tables is also the responsibility of these bodies, concerning both the health aspects established by legislation and the nutritional aspects.

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