Natural food additives: *Quo vadis?*

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Abstract
In a time where the public is more aware and interested with what they eat, natural additives have been gaining interest both from the food industries and the consumers. Some studies show that consumers prefer food prepared with natural additives rather than chemical ones, due to health reasons. Although quite promising, natural additives still face some drawbacks and limitations as well as conflicting information. In this manuscript, the most important natural additives are overviewed, as well as their use, benefits and risks. The future of these molecules along with new types of additives are also summarized.

Keywords: natural food additives, antimicrobials, antioxidants, sweeteners, colorings

Food as a basic need
The importance of food for mankind is undeniable; there is still no way of living without eating, therefore, this commodity is of utmost importance for the well-being of every man, woman and child across the world. Although the need to feed has maintained itself immutable across the ages, the way we consume foodstuffs has seen deep changes. From the local gatherers in the Paleolithic to the domestications of animals and vegetables there was a huge leap, only surpassed by the commercial trading of spices and other goods in the fifteenth century. Today, in modern countries food is produced in specific facilities and then transported to markets that can be within the same country or even in distant ones (Atkins and Bowler, 2001). Delivering food in good conditions from the production site to the consumer requires a great load of energy, either by refrigeration, controlled packaging or the use of additives to avoid spoilage and reduce food alteration. In a competitive global market, the least expensive method of food preservation is always favored, and in most cases, food additives are
chosen over the others. Furthermore, food additives are essential to enable the food industry to make food meet the increasingly challenging market and legal demands (Saltmarsh et al., 2013).

**Food additives**

The Codex Alimentarius defines a food additive as “any substance not normally consumed as a food itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants, or substances added to food for maintaining or improving nutritional qualities, or sodium chloride” (Codex Alimentarius; Motarjemi, Moy and Todd, 2014). The Food and Drug Administration of the United States (FDA) defines a food additive as “any substance the intended use of which results or may reasonably be expected to result -- directly or indirectly -- in its becoming a component or otherwise affecting the characteristics of any food. This definition includes any substance used in the production, processing, treatment, packaging, transportation or storage of food. The purpose of the legal definition, however, is to impose a premarket approval requirement. Therefore, this definition excludes ingredients whose use is generally recognized as safe (where government approval is not needed), those ingredients approved for use by FDA or the U.S. Department of Agriculture prior to the food additives provisions of law, and color additives and pesticides where other legal premarket approval requirements apply” (FDA). The European Food Safety Authority’s
(EFSA) definition for food additive is “any substance not normally consumed as a food itself and not normally used as a characteristic ingredient of a food, whether or not it has nutritive value, the intentional addition of which to a food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may reasonably be expected to result, in it or its by-products becoming directly or indirectly a component of such food” (EFSA, 2008; Saltmarsh et al., 2013). Food additives could be divided into 6 groups of molecules: preservatives, nutritional additives, coloring agents, flavoring agents, texturizing agents and miscellaneous agents. Furthermore, the preservatives are sub-divided into antimicrobials, antioxidants and antibrowning agents; the coloring agents encompass the azo compounds, the chinophthalon derivatives, the triarylmethane compounds, the xanthenes and the indigos; the flavoring agents include the sweeteners, the natural and synthetic flavors and the flavor enhancers. Finally, the texturizing agents are divided into emulsifiers and stabilizers (Carocho et al., 2014).

The spread of food additives reaches all kinds of foods, since the minimally processed until the highly processed and transformed foodstuff. The interaction between some food additives and the general public has not been peaceful. In the 80’s food additives were considered dangerous to be consumed, which fueled generalized scares and removal of some additives, namely colorants from processed food. Since then, the relation between additive and consumer has improved, although some distrust still lingers (Emerton and Choi, 2008). Today, some authors report health issues on the consumption of food additives, even though the authorities periodically review the data supporting the safety and correspondent acceptable daily intake (ADI). Books like “What’s really in your basket” (Statham, 2007) review all the food additives and divide them into safe, caution advised and finally best avoid or hazardous. While this author
does not show the data to support his claims, other authors study individual additives or groups of additives and show evidence of the threats that some of them can pose. Regarding the antimicrobials used in food, the most widespread are benzoates, sorbates, propionates, nitrites and parabens. Although studied for decades, some potential dangerous effects towards health are still found for many of them. Sodium benzoate, although regarded as safe, has yet to prove that it is not hazardous on long term exposure (Lennerz et al., 2015). Depending on the dose used, sodium sorbate proves to be genotoxic on in vitro blood lymphocytes (Mamur et al., 2010), parabens have been proved to induce migratory and invasive activity in human breast cancer cells in vitro, while their dermic exposure has been overlooked, proving that this type of exposure can be added to the oral exposure, therefore increasing the overall intake (Karpuzoglu et al., 2013; Khanna et al., 2014). Extensive studies have been carried out through the years regarding sorbates and their health implications. Some studies describe these compounds as genotoxic and mutagenic, while others refer to this not being relevant. Still, controversy lingers, due to legislation, in which sodium sorbate is not allowed in the US, but legal to be used in food in the EU (Binstok et al., 1998; Mpountoukas et al., 2008; Mamur et al., 2012). Nitrates (E240-E259) and nitrites (E249-E250) are other antimicrobials that are used in foodstuffs. Nitrates have recently been restricted within the EU, and can now only be added to meat for slow curing. Nitrites are used in meat for color formation, flavor enhancement and antimicrobial activity, being the only food additive to inhibit the botulinum toxin. They are also allowed in pickled herring, sprat and ripened cheese. Its use in the EU has been approved at the minimum possible dosage. Nitrites can be found in nontreated fruit and vegetables, and can take part in the formation of nitrosamines (Gotterup et al., 2007; Sebranek and Bacus, 2007; Honikel, 2008; Watson and Preedy, 2010; EU Reg. 1129/2011; Sindelar and Milkowski, 2012;
Nitrites have been shown to have carcinogenic effects, among other deleterious effects towards humans, namely the oxidation of oxyhemoglobin to ferrihemoglobin (Cammack et al., 1999). Sulphites or sulphiting agents are used in food like wine, dried fruits, dehydrated biscuits, fish, among others, to avoid antimicrobial contamination, to excerpt antioxidant and antibrowning activity. They are known to have cytotoxic and carcinogenic effects towards both rats and humans (Suh et al., 2007; Iammarino et al., 2012).

Among the synthetic antioxidants, butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin, tert-butylhydroquinone and propyl gallate are the most common. Many studies have been carried out regarding these compounds, and while some studies point out hazardous effects such as toxicity and carcinogenic effects, others show the opposite, regarding them as tumor supressant (Ikezaki et al., 1996; Botterweck et al., 2000; Bauer et al., 2001; Carocho and Ferreira 2013a; Vandghanooni et al., 2013).

Colorings like indigocarmine are found to be dangerous by producing superoxide dismutase during metabolisation in mice (doses between 1 µM and 100 µM) (Kohno et al., 2005). Safflower yellow and Kokum red have shown to have clastogenic effects in mice bone marrow (Agarwal et al., 1994). Tartrazine, a widespread food colorant has been linked to irritability, restlessness and sleep disturbance in children (Rowe and Rowe, 1994). Many studies also deem colorings safe, within the ADI, but a study has proven that children could be consuming a higher quantity of dyes that initially thought, while adults are also exposed to other means of entrance into the body are overlooked (e.g. shaving creams and after shave products) (Lucová et al., 2013; Stevens et al., 2014). Finally, among the sweeteners, mainly the “intensive sweeteners”, such as saccharine, aspartame, sucralose and acesulfame K are the most common and widespread used in food industry, mainly in low caloric food products. All of them
provide the sweetening power at low doses. Saccharine and sucralose as regarded as safe to consume with restrictive maximum level (EU Reg. 1129/2011), aspartame still poses some controversial effects, namely by having deleterious effects on human babies during gestation, and by causing oxidative stress in wistar albino rats (Choudhary and Rathinasamy, 2014; Toigo et al., 2015). Acesulfame K has been proved to have clastogenic effects in mice and to induce allergies in humans (doses between 15 and 2250 mg acesulfame Kg/body weight) (Mukherjee and Chakrabarti, 1997; Stohs and Miller, 2014). Many more reports of the dangers of consuming synthetic food additives can be found in the available literature (Carocho et al., 2014). The concern of the ADI of synthetic additives their overconsumption has always been of great importance for the governing bodies. Back in 2001, the EU published a report regarding some member states, informing the consumption of additives in relation to their ADI. Although at the time, only preliminary results were given, and many additives were consumed under their ADI, some additives were clearly being overconsumed by the European population, namely, sulphites (E220-228), nitrites (E249-250), polysorbates (E432-436), sucrose esters and sucroglycerides (E473-474), stearoyl-2-lactylates (E481-482), sorbitan monolaureate and sorbitan monooleate (E493-494), aluminium sulphates (E520-523), sodium aluminium phosphate (E541) and aluminium silicates (E554-556/559). These additives were reported as being consumed in excess by the adult population, while many more were reported being consumed in excess by children. Although this report insisted that new ones should be carried out in the following years, including all the members of the EU and with more accurate data being gathered, this did not occur (Commission Report, 2001). When the EFSA was formed, in 2002, it preferred to review the consumption and ADI estimations individually based on scientific committees and panels, and has been doing this until today. Many food
additives have been re-evaluated by these panels and in some cases the ADI has changed, being all these reports published in the EFSA official journal and website. Furthermore, in 2010, concerned with food additives consumption, EFSA started a re-evaluation of all food additives gathering more studies and expert opinions, in order to find possible health implications and to adjust their ADI. This evaluation is gradual and is expected to end by 2020 (Carocho et al. 2014).

Natural Additives – Shedding some light on the controversy of food additives or creating more entropy?

For some decades now, natural food additives have been gaining more interest both from the public and food manufacturers. Generally, the public will choose a food with no additives, but if these are not available, the same consumer will chose, if possible, a food containing natural additives over synthetic ones (Carocho et al., 2014). Depicted in Figure 1 are the main categories of food additives along with the most used compounds of each category. In Table 1, all the studied additives are represented, along with the foods or foodstuffs they are used in. The endless amount of both synthetic and natural additives, added to the lack of knowledge of most of the population when it comes to distinguishing natural from synthetic compounds does not help to clarify what is what in the label of most food products. The only way to overcome this limitation is to provide consumers with valuable information regarding these compounds purposely added to food.

What are natural additives?

Consumer studies have shown that consumers have recently become more informed about food additives and always tend to choose the additives of natural origin than their
synthetic analogues (Devcich et al., 2007; Pokorný, 2007; Bearth et al., 2014). Surprisingly there is no definition of natural preservatives, antioxidants, colors or sweeteners. Only natural flavorings have legislation both in the EU and the USA, and this is then transposed to the other classes of additives, leading to wrong interpretations and the confusion of what is natural or synthetic. There is a growing need for transparent legislation regarding the natural additives, for they are of growing interest in developed countries (Baines and Seal, 2012).

**Classes of natural additives**

There are no defined categories for natural additives; in the EU, they are incorporated into the same “E” classification as all the other counterparts (Council Regulation (EC) 1129/2011). Still, the most researched natural additives are antioxidants, antimicrobials, colorings, and sweeteners, which are revised in this manuscript (**Figure 1**).

**Natural antioxidants**

Antioxidants are mainly used in food to prevent off-flavors by oxidation of fats, therefore halting their peroxidation in the initiation or propagation phases. There are 5 types of antioxidants; the primary antioxidants known as radical scavengers or chain-breaking antioxidants; chelators, that bind to metals and prevent them from initiating radical formation; quenchers, which deactivate high-energy oxidant species; oxygen scavengers, that remove oxygen from systems, avoiding their destabilization; and finally the antioxidant regenerators, that regenerate other antioxidants when these become radicalized. The main foods where antioxidants are used are meats, oils, fried foods, dressings, dairy products, baked goods and extruded snacks (Baines and Seal, 2012).
Polyphenols are some of the most interesting groups of natural compounds in the vegetable kingdom, and due to their strong antioxidant capacity they display interesting effects towards human health, namely against cancer, osteoporosis, cataracts, cardiovascular dysfunctions, brain diseases, and immunological conditions (Carocho and Ferreira, 2013b). Due to their high efficacy in preserving food and their wide acceptance from the general public, it is desirable to add them to food. In all the classes of polyphenols (phenolic acids- hydroxybenzoic or hydroxycinnamic acids, flavonoids including anthocyanins, tannins, lignans, stilbenes, and coumarins), some stand out with higher potential than others. They can be added as plant extracts, taking advantage of the synergistic effects between compounds, or by further purification to individual molecules, adding the most bioactive ones to the foodstuff. Polyphenolic extracts like rosemary and other extracts from plants have been used to act as antioxidants in food and, in terms of rosemary, it has been identified as a food additive in the Council Regulation (EC) 1129/2011, with the E number 392. Although the synergistic effect between the compounds are important for the extracts antioxidant activity, some industries seek specific molecules to carry out these effects. Carnosic acid, a hydroxybenzoic acid derivative, is a known constituent of rosemary extract and is believed to have the most important antioxidant effect in it. It is used in oils, animal fats, sauces, bakery wares, meat patties (between 22.5 and 130 ppm for the meat patties) and fish, among others (Naveena et al., 2013; Bitrić et al., 2015). Ferulic acid, a hydroxycinnamic acid, is also used in the food industry as an antioxidant and a precursor of other preservatives, as well as taking part in food gels and edible films (Kumar and Pruthi, 2014; Ou and Kwon, 2004). Catechin, a widely known flavon-3-ol, is also known for its antioxidant activity. It can be directly added to food, joined with other natural substances and even encapsulated to promote and extend its effects.
Regarding other compounds with potential antioxidant activity, we could stand out the ascorbic acid, also known as vitamin C, is a high oxygen scavenger used in various foodstuffs. It regenerates phenolic oxidants and tocopherols that have suffered oxidation, due to its higher oxidation potential, as explained in **Figure 2**. Ascorbic acid (E-300) is particularly important to stabilize lipids and oils, but can be used in other matrices. In 2015, the EFSA gathered a scientific opinion regarding ascorbic acid and determined there was no risk in its consumption, not defining an ADI (Baines and Seal, 2012; EFSA, 2015a). Carotenoids are also known for their antioxidant potential as food additives, although their use is always limited by being very susceptibility to oxidation by light. Lycopene (E-160d) is the most abundant carotenoid, being found mainly in tomatoes, although it is not widely used as a food antioxidant. On the other hand, β-carotene is used in baked goods, eggs, and dairy products, among others, as a singlet oxygen quencher (Smith and Hong-Shum, 2011). In many foodstuffs that use carotenes, ascorbic acid or vitamin E (tocopherols) are used to benefit from synergies. Carotene mixes and β-carotene have been reviewed by the EFSA’s scientific panel and ruled out any toxicity arising from its consumption, whether from synthetic provenance or extraction from plants and fruits (EFSA, 2012).

Tocopherols, which are the building blocks of vitamin E, are also known as very strong antioxidants. They can act isolated or in synergy with ascorbic acid, by regenerating it (**Figure 2**). Apart from this, their main antioxidant function is by terminating free radicals in autoxidation reactions (Smith and Hong-Shum, 2011). In some cases tocopherols are used in films and coatings (Barbosa-Pereira et al., 2013; Lin and Pascall, 2014; Marcos et al., 2014), although they can be used as an additive as well (E-
These compounds have been used in bacon (300 mg/kg), meats, dairy products and oils, among others (Smith and Hong-Shum, 2011; Wang et al., 2015).

**Natural Antimicrobials**

Natural antimicrobials are also a very hot topic for food processing; they guarantee that the food is free of microorganisms and safe to eat. There are natural antimicrobials of three sources, derived from microorganisms, from animals and from plants. Ideally all natural antimicrobials should have a broad action, have bactericidal and fungicidal activities rather than only inhibitory ones, active at low concentrations, heat stable, unaffected by pH, impart no flavor or color, have no toxicity, easily assayable, have no pharmaceutical application, not susceptible to resistance from contaminants, label friendly, and finally cost effective.

Antimicrobials derived from microorganisms are molecules resulting from living organisms that have impact on others. Examples of this are bacteriocins; to date, about 300 bacteriocins have been discovered, with some of them having the potential to inhibit the growth of other proteins. There are three types of bacteriocins, class I, class II and bacteriolysins; in which the first class contains lanthionine, the second class is non-containing and the third are non-bacteriocins lytic proteins (Cotter et al., 2005; Hammami et al., 2007). The main limitation of bacteriocins is the limited microbial species that are affected by them, which tend to be species very closely related to each other. Nisin, one of the most used bacteriocins, has a broad spectrum of action in terms of the species it is able to inhibit. It is used both as a food additive (E-234) and as a constituent of coatings and films. It is applied to dairy products (from 100 to 4000 IU/mL), beverages, eggs, meat (from 400 to 1000 IU/mL), among others. Recent studies suggest that it can be potentiated by synergisms with other antimicrobials (Cleveland et
Another bacteriocin is pediocin, which is also used as an antimicrobial against the dangerous *Listeria* microorganisms. It can be used as a film to coat sliced ham, but can also be extended to dairy products (Santiago-Silva et al., 2009; Lacroix, 2011). These two proteins, nisin and pediocin, are the most widespread and commercially used bacteriocins, although others like sakacin, which is rather a group of bacteriocins, are used in cooked ham and cold cuts (from 12 to 35 µg g\(^{-1}\)) (Katla et al., 2002; Jofré et al., 2007). The application of bacteriocins has been gaining interest in the food industries due to their natural origin and the benefits that can be achieved with their incorporation into foodstuffs, although pediocin and sakacin are not considered a food additive in the EU.

Natamycin is also a widely used natural preservative. It is a polyene macrolide with antifungal activity, especially active against yeasts and moulds and virtually without effect on bacteria, protozoa and viruses. It has been used in a variety of foodstuffs, both as a free additive (E-235), encapsulated and as a constituent of films (Roller, 2003; Baines and Seal, 2012). It has been used to control the growth of yeasts in cheese (from 2.31 to 9.25 mg/dm\(^2\) film) (Oliveira et al., 2007; Resa et al., 2014b), as a constituent of chitosan based films in Saloio cheese (from 0.0625 to 2 mg mL\(^{-1}\)) (Fajardo et al., 2010). Furthermore, beverages are also included in the application of this macrolide (Roller, 2003). In 2009, the EFSA asked for a scientific opinion regarding the use of Natamycin as a food additive and concluded that the exposure is very low, and that the very poor absorption of this molecule by the human body rules out any risk of its use (EFSA, 2009). Reuterin is an antimicrobial compound produced by the Gram positive lactic bacteria *Lactobacillus reuteri*, being effective against *Listeria* species. Reuterin has application in food by adding the *Lactobacillus* into the foodstuff as starter cultures with
glycerol, namely in cheese (Langa et al., 2013; Gómez-Torres et al., 2014), or by application of reuterin after extraction. It has been used to coat the surface of sausages and cold-smoked salmon (Kuleaşan and Çakmakçi, 2002; Montiel et al., 2014) and also by incorporation into cottage cheese (Lacroix, 2011). Poly-L-Lysine is a homopolimer of the amino-acid lysin that has a GRAS (generally regarded as safe) status in the US and is allowed as a natural food additive in Japan, where it is used in staple foods for many years (Shih et al., 2006). Large amounts of this additive may confer a bitter taste to food, but due to its high antimicrobial effect, very low quantities are required. Furthermore, synergistic effects with other natural antimicrobials enhance its capacity (Yoshida and Nagasawa, 2003). The applications of this compound are related with outer protection of fish and sushi, along with preservation of rice, noodles, vegetables, soups, salads, steam cakes and custard creams (Baines and Seal, 2012).

Antimicrobials derived from animals are compounds like proteins and enzymes that are isolated from animals or are animal derived. Today, the only authorized natural antimicrobial derived from animals is lysozyme, which is used both in the US and the EU (E-1105). The lysozyme used is derived from eggs (Baines and Seal, 2012). This enzyme’s antimicrobial activity relies on the hydrolysis of the β-1,4 linkage site of the peptidoglycan in the bacterial walls, therefore yielding very high activity against Gram negative bacteria (which is constituted of 90% of peptidoglycan) and moderately effective against Gram positive bacteria (with much less peptidoglycan), but with no action against yeasts or fungi (Barbiroli et al., 2012). The main commercial use of this natural biocide is in the cheese industry, where it is added to avoid “late blowing” of cheese, although studies have been carried out in eggs, milk (2mg/mL in 25 mL milk) and beef (200 mg/90mg beef) (Sung et al., 2011). Lysozyme has also been assayed to take part in biofilms and edible coatings (Appendini and Hotchkiss, 2002; Barbiroli et
al., 2012) while synergistic effects with other natural antimicrobials have also been researched (Bayarri et al., 2014). Another natural antimicrobial compound is the enzyme lactoperoxidase, which belong to the peroxidase-cyclooxygenase superfamily and is very abundant in bovine milk. The antioxidant activity is carried out through the lactoperoxidase system, consisting of lactoperoxidase, thiocyanate and hydrogen peroxide. The antimicrobial activity relies on the oxidation of thiocyanate, in which the intermediate compounds formed have antimicrobial activity (Batt and Tortorello, 2014). The main use of lactoperoxidase is to maintain raw milk, especially in places where refrigeration is not readily available. By adding thiocyanate to the milk, the lactoperoxidase system will start, displaying its antimicrobial activity. This addition is necessary, due to amount of thiocyanate present in the milk not being enough to trigger the antimicrobial activity. Newer applications have been suggested for this enzyme, such as preservation of fruit juices (Touch et al., 2004), and also as coating of foodstuffs (Cissé et al., 2015). Lactoferrin, a GRAS, iron binding glycoprotein very abundant in milk also displays antimicrobial activity. Although there are few reports of its use, researchers claim that it is potentially a molecule that can be used in the future to control microbes in food (Davidson et al., 2005; Baines and Seal, 2012). The oldest reports of its use are in baby formulas, although it is now used as a spray on meat to prevent contamination (Naidu, 2002; Wakabayashi and Takase, 2006; Baines and Seal, 2012).

Antimicrobials derived from plants are usually compounds belonging to their secondary metabolism, which confer protection from predators, code for signalling molecules and help the plant resist stress. Examples of compounds from this metabolism are terpenes, steroids, alkaloids and polyphenols. The polyphenols group encompasses various classes of molecules, and although they are attributed to have many biological effects
on health, there are only few reports of some polyphenols with application in coatings and films showing antimicrobial activities (Sun et al., 2014; Giteru et al., 2015; Kaewprachu et al., 2015). Another very important group of molecules with antimicrobial activity are the essential oils. These compounds are complex mixtures of volatile compounds produced by living organisms. The most used essential oils are isolated through physical means from plants, and derive from the mevalonate, methyl-erithrytol and the shikimic pathways, with each one yielding different compounds (Başer and Buchbauer, 2010). Out of the 300 known essential oils of different plants, some have found their way into foodstuffs due to their antimicrobial activity; in fact, some have achieved the GRAS label in the US. Of these, oregano oil, thymol, carvacrol, clove oil, cinnamon oil and clove oil are some of the most important. There is a vast number of foodstuffs where essential oils have been applied, namely meat, fish, dairy products, vegetables, rice and fruit (Burt, 2004). Furthermore, recent developments have also been done in the packaging, with some films being impregnated with essential oils, namely with carvacrol and thymol (Ramos et al., 2012).

**Natural Colorants**

Colorants are used in food to make it feel more appealing and appetising, which are important factors when choosing food off the shelves. The colorants are used to enhance existing colors that can be lost either during the manufacture or over the shelf life, or even to attribute new ones to it. Food colors can be classified into three groups; natural food colorants, which refer to ones that are synthesized naturally; nature-identical colorants, which although synthesized in industries, mime the natural ones and finally the artificial/synthetic colorants (Msagati, 2013). There are many colorants used in the food industry, and even the natural or nature-identical represent an important amount,
with some already allowed to be used and legislated. Annatto is a permitted natural food colorant, extracted from the *Bixa Orellana* L. tree, with the E number E160b. The main constituents of the annatto mixture are the carotenoids bixin and norbixin, which display a yellow to orange coloration. There are many foodstuffs where annatto is used, cakes (from 250 to 1000 mg/kg of dough), biscuits, rice, dairy products, flour, fish, soft drinks, snacks and meat products (Hendry and Houghton, 1996; Rao et al., 2005; Scotter, 2009). Paprika is another mixture of two carotenoids, capsanthin and capsorubin, it is also approved in the EU (E160c) and displays an orange to red color (Hendry and Houghton, 1996). There are many other carotenoids used in food namely β-carotene, lutein, violaxanthin, neoxanthin, β-cryptoxanthin, fucoxanthin, lycopene and astaxanthin. They are extracted from plants, algae and even insects and represent a wide spectrum of colors in the food industry. The main applications of carotenoids in food are related to sauces, marinades, spice blends, coatings, beverages, milk, among others (Baines and Seal, 2012). Anthocyanins (E 163) are responsible for pigments in nature, namely red, purple, violet and blue and this can be transposed to food when they are used as colorants. The main anthocyanins in nature are cyaniding, delphinidin, malvinidin, pelargonidin, peonidin, petunidin, being their main applications in soft drinks, confectionary products and fruit preparations (Hendry and Houghton, 1996; Baines and Seal, 2012). In 2013, the EFSA required a scientific opinion on the safety of their consumption as a food additive, which concluded that further research should be carried out due to a lack of toxicological data (EFSA, 2013). Other very similar compounds to anthocyanins are betalains, which display colors ranging from red-violet (betacyanins) to yellow-orange (betaxanthins). They are not exhaustively studied like anthocyanins, but still have some applicability in the food industry as natural colors due to having three times more coloring strength than anthocyanins (Stintzing and Carle, 2012).
The only betalain legislated for use is derived from beetroot (E 162-betanin), and has application in dairy products, meat products and many others (Delgado-Vargas et al., 2000). Chlorophylls (E 140) are vegetable pigments that occur naturally in plants and confer color. Among the five different chlorophylls that exist, only two (a and b) are used in the food industry as colorants. Their complex structure is difficult to stabilize, being this the main drawback of their use in the industry, which has studied mechanisms of retaining or replacing the magnesium ion within the structure. The used commercial colorants of chlorophylls are extracted from alfalfa, and have been employed in dairy products, soups, drinks and sugar confections (MacDougall, 2002). Furthermore, in 2015 a panel of experts ruled out any toxicological implication of these pigments due to their presence being much higher in the diet than as an additive (EFSA, 2015b). Curcumin (E 100), a pigment purified from turmeric which is extracted from the dried rhizomes of the plant Curcuma longa L., is another widespread used food colorant. It confers an orange color to food, and is used in mustard, yoghurt, baked goods, dairy industry, ice creams and salad dressings (Hendry and Houghton, 1996; MacDougall, 2002). This additive was under review by the EFSA due to possibly being over the ADI, but in a statement issued by this same organization, after carefully reviewing the available data, it was determined that the consumption was under the ADI (EFSA, 2014a). Carminic acid (E 120) is the main pigment present in the insect Dactylopius coccus Costa, which when complexed with aluminium, renders a brilliant red color. This coloring agent is quite expensive when compared to other natural red ones, like anthocyanins, although it is considered technologically important due to its stability. It is used in jams, gelatins, baked goods, dairy products and non-carbonated drinks (MacDougall, 2002).
Natural Sweeteners

Sweeteners have been used for centuries to make foodstuffs more appetizing and appealing to consumers. Sweeteners were first introduced due to the high caloric contribution of sugar to the diet, fostering obesity in the population, which was quite prevailing in infants and children. Thus, in the eighteen hundreds a low-calorie sweetener became available, saccharine. Then, with the success of this sweetener, others followed, namely cyclamates, aspartame and acesulfame K, which are the most widespread. Sweeteners have also been targets of scandals and controversy throughout the years, with allegations of carcinogenicity, foetus malformations, toxicity to liver and bladder, among other dangers. Although many of these allegations have been researched and the sweeteners have been deemed safe, a certain distrust among consumers still lingers, adding to the fact that some are not allowed to be used in the USA and other in the EU (e.g. cyclamic acid and cyclamates, which are not allowed in the US and are allowed in the EU under the E number 952), the search for natural alternatives is imperative (Carocho et al., 2014). Natural sweeteners have the same objective as synthetic ones, to deliver a sweet taste while contributing with less or no calories at all to the diet. Natural sweeteners can be divided into two groups, bulk sweeteners and high-potency sweeteners. The former have a potency of one or less sucrose molecule (sucrose is the international standard for sweetness), while the latter have a potency which is, higher than the sweetness of one sucrose molecule. For natural sweeteners, to be considered viable to be introduced in the markets and widely used, they need to have a good taste, be safe, have a high solubility, a high stability and an acceptable cost-on-use (Baines and Seal, 2012). In this manuscript, only the natural sweeteners that fulfil all these criteria are revised. Regarding the bulk sweeteners, the two main compounds of this group are erythritol and tagatose. Erythritol (E 968) is a sugar alcohol (polyol),
which occurs naturally in some fruits and vegetables, although it is industrially produced through enzymes and osmophilic yeasts or fungi. It was first purified in 1848 making it one of the oldest natural sweeteners available. It is allowed both in the US and in the EU although in the latter with some restrictions in beverages. Being a bulk sweetener it only has around 65% of the sweetness of sucrose, but does not cause tooth decay or is toxic or carcinogenic in the quantities used in food. The main foodstuffs where erythritol is used are baked goods, coatings, frostings, fermented milk, chocolate, low-calorie beverages, candy, chewing gums, among others (O’Brien-Nabors, 2001; Baines and Seal, 2012). In 2014, a scientific panel required by EFSA, ruled out its laxative effects, and considered it safe for consumption without specifying an ADI (EFSA, 2015c). Tagatose is a ketohexose, an enantiomer of fructose, and is also considered a prebiotic and a flavor enhancer. It occurs in very small quantities in fruits and heat treated dairy products. Its potency in relation to sucrose is 92%, which makes it very similar in terms of taste although only contributing with 1.5 Kcal g⁻¹, making it safe for diabetics to consume while not being hazardous to teeth. The industrial production of tagatose is derived from lactose with a number of enzymatic steps, along with fractionation and purification. Tagatose is approved in the US as a GRAS compound and is also allowed in the EU as food ingredient and many other countries with virtually no toxicity associated to its consumption. The applications of tagatose in the food industry encompasses cereals, beverages, yoghurts, frostings, chewing gum, chocolate, fudge, caramel, fondant and ice cream (O’Brien-Nabors, 2001; Dobbs and Bell, 2010; Baines and Seal, 2012).

Regarding high potency sweeteners, steviol glycosides (E 960) (EFSA, 2014b) are an example of natural compounds with a high dissemination around the world. These glycosides, mainly steviosides and rebaudiosides are also know just as stevia, stevioside
or steviol, and are purified from the plant *Stevia rebaudiana* Bertoni. Due to having various compounds in its formula, steviol glycosides have different potency, with the lowest ones being 30 times sweeter than sucrose (dulcoside A, rebaudioside C) and others which are about 300 times more potent (rebaudioside A). There is no industrial way to produce these glycosides and their production relies on the harvest from the herb, which fostered a high production of these plants, especially in China. Steviol glycosides have been approved as a sweeter in many countries, including the EU and USA, with great results regarding toxicity, cariogenicity, carcinogenicity and allergic reactions. Among the food industry, steviol glycosides are used in beverages, dairy products, ice cream, frozen desserts, sugar-free confectionary, mints, dried sea-foods and sauces. The representation of the extraction method of steviol glycosides is displayed in Figure 3 along with the relative percentage of all glycosides present in the mix. In 2014 steviol glycosides were researched by a scientific panel of the EFSA and determined that, despite previous allegations, the consumption of these molecules was under the ADI and did not pose a toxicological threat as a food additive (Brandle et al., 1998; O’Brien-Nabors, 2001; Brusick, 2008; Baines and Seal, 2012; EFSA, 2014; Urban et al., 2015). Another high potency sweetener is glycyrrhizin (E 958) (Barclay et al., 2014), a triterpene glycoside extracted from *Glycyrrhiza glabra* L., the liquorice plant. This compound, also known as glycyrrhizic acid can act as a sweetener with a potency of 50 times sweeter than sucrose, but also as a foaming agent and flavor enhancer. This compound is legally used in the US and EU under the form of monoammonium glycyrrhizinate and ammoniated glycyrrhizin. It is manly used in liquorice, baked goods, frozen dairy products, beverages, confectionery and chewing gum (O’Brien-Nabors, 2001; Spillane, 2006; Baines and Seal, 2012). Thaumatin (E 957), a mixture of five proteins (taumatin I, I, III, a and b) are also used as a sweetener
in many countries. Thaumatin is extracted from the fruit of Thaumatococcus daniellii Benth, a plant native to Africa. There is no conclusive value of its potency; some authors consider it to be about 1600 times stronger than sucrose, while other point out values above 3000. Due to its liquorice cool aftertaste, thaumatin is not used at high quantities, although it is very useful to mix with other sweeteners to confer umami taste and to reduce bitterness in foodstuffs. The main foods where it is employed as either a sweetener or flavor enhancer are sauces, soups, fruit juices, poultry, egg products, chewing gum, processed vegetables, among others (O’Brien-Nabors, 2001; Baines and Seal, 2012). Neohesperidin dihydrochalcone, a widely used sweetener in both the EU (E959) and the US (GRAS), which is synthesized from neohesperidin or naringin is considered synthetic, although its origin is from compounds of citrus fruits. It is 1500 times more potent than sucrose and is used in juices, jams, chewing gum, confectionary and milk, among many others (O’Brien-Nabors, 2001; Spillane, 2006; Baines and Seal, 2012; El-Samragy, 2012). There are some other natural sweeteners that could be used in the future, but do not have any applications in foodstuffs today. Examples of these compounds are monatin and brazzein. This is due to their scarcity and poor yields when isolated from plant matrices.

**Concluding Remarks**

Natural additives have come a long way from their beginnings as archaic additives to becoming in some cases the leading manner of conserving food. The controversy and ambiguity among the chemical additives allied to the sporadic scares have paved the way for natural additives to gain interest and funding. Today, most consumers will prefer natural additives to be added to food, rather than synthetic ones, which is seen by the food industry as an opportunity to find new and more efficient natural ones, while
also fighting to reduce the overall addition of additives, producing minimally processed goods. The benefits of natural additives are endless, their synergy and potency is a great leap from synthetic additives that carry out, in most cases, only one effect over the food. Although not all natural additives are produced in the same way (extraction from plants or microorganisms, purification, enzymatic alterations) they tend to be safer than the synthetically produced ones, but toxicity is always a detail that must not be overlooked. Toxicological, carcinogenic and other safety studies should be carried out on these additives in order to ensure their safety and avoid scares. Limitations still linger when it comes to natural additives, while some are the same as with synthetic ones, others are specific to their natural provenance. For one, the price of natural additives must be reasonable when compared with the synthetic compounds that carry out the same effect, or else they will not be considered as an option for food due to the competitive globalized market. Another great limitation is the real effectiveness of natural additives; this is important due to the quantity that is added to food. If the potency is weak and a great quantity is needed, this might alter the foodstuff completely in terms of appearance, taste or texture, therefore, the potency vs effective outcome must be properly attained. Another drawback closely related to this one is the fact that some natural additives are needed in higher quantities than synthetic ones, and in some cases it is not profitable or advisable to use. Legislation also poses great challenges to natural additives due to the lack of separate legislation for natural additives, which are legislated in the same manner as synthetic ones, sometimes not being clear how they are produced or where their source lies (synthetic or natural), causing more entropy in the consumers perception. The delay on new additives approval is transversal to both natural and synthetic additives, and does not favour the introduction of new compounds in the market. This can be in part explained by the exhaustive assays that have to be
carried out to determine a safe dosage, rule out interactions, allergens or hypersensitivity as well as to reach an ADI. Furthermore, the legislation regarding additives is not transversal across countries, and the labels of products has to change to be in agreement to the local legislation, which fosters controversy and lacks simplicity, which is desirable when it comes to choosing food from the shelves. A specific problem with certain natural additives is the difficulty to find sources of the plants or microorganisms that produce them, causing in some cases an over harvesting which can lead to harm for the ecosystems they are inserted in. The synergistic effects and the capacity to carry out many functions at the same time is quite normal in natural additives, but some are not compatible with other natural or synthetic ones, as well as with ingredients in the food itself, which in some cases excludes them from being used. Despite these limitations, natural food additives are the future of food preservation due to the health benefits and synergies, and will gain even more interest in the future with the limitations being solved. The consumption of these compounds has been encouraged in the past years, with new products displaying labels of “all natural additives” or “no synthetic additives”. An increasing amount of funding is being channelled to find better, safer and more efficient natural additives. Finding these ultimate additives is the challenge of the food industry for the near future. These compounds would be, without a shadow of a doubt, safe, cheap and would not interfere with the food. Although hard to achieve, we have been much further from finding it. Until then, we must rely on the slow and not always clear advances of science, which should be hands in hands with legislators and not in conflict with them, leading to healthier and safer food for the consumers worldwide.
Acknowledgments

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Conflict of Interest

The authors state no conflict of interest regarding this manuscript.

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polyesters containing $\alpha$-tocopherol and olive leaf extract for food packaging applications. *Food Packaging and Shelf-Life*, 1, 140-150.


Natural antioxidants
- Polyphenols
- Ascorbic acid
- Carotenoids
- Tocopherols

Natural antimicrobials
- Bacteriocins
- Natamycin
- Reuterin
- Poly-L-Lysine

Natural colorants
- Annatto
- Paprika
- β-carotene
- Lutein

Natural sweeteners
- Erythritol
- Tagatose
- Steviol glycosides
**Figure 1.** Depiction of the most common additives divided by categories. Represented in orange are the antimicrobials derived from microorganisms. In blue, the antimicrobials derived from animals and in green the ones derived from plants. The purple color represents bulk sweeteners and in yellow the high potency ones. In black, the antioxidants and colors derived from plants.

**Figure 2.** Representation of lipid peroxidation and regeneration of α-tocopherol by ascorbic acid. Adapted from Carr et al., (2000).
Figure 3. Representation of the extraction method of Steviol glycosides from the leaves of *Stevia rebaudiana* (Bertoni), along with the relative percentage in dry weight of the different glycosides. Adapted from Ceunen and Genus, 2013; FAO, 2007.
Table 1. Representation of the studied additives, along with the foods they are used in.

<table>
<thead>
<tr>
<th>Additive</th>
<th>Use in Food and/or Foodstuffs</th>
<th>GRAS status / EU E number</th>
<th>Reference</th>
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<td><strong>Antioxidants</strong></td>
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<tr>
<td>Carnosic acid</td>
<td>Oils</td>
<td>Not GRAS / no E number</td>
<td>Naveena et al., 2013  Bitrić et al., 2015</td>
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<td>Animal fats</td>
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<td>Bakery wares</td>
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<td>Fish</td>
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<td>Ferulic acid</td>
<td>Gels</td>
<td>Not GRAS / No E number</td>
<td>Ou and Kwon, 2004  Kumar and Pruthi, 2014</td>
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<td>Edible films</td>
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<td>Catechin</td>
<td>Encapsulation</td>
<td>Not GRAS / No E number</td>
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<td>β-carotene</td>
<td>Bakes goods</td>
<td>GRAS / E160a</td>
<td>Smith and Hong-Shum, 2011</td>
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<td>Tocopherols</td>
<td>Films</td>
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<td><strong>Antimicrobials</strong></td>
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<td>Nisin</td>
<td>Coatings</td>
<td>Not GRAS / E234</td>
<td>Cleveland et al., 2001  Millette et al., 2007  Nguyen et al., 2008  Lacroix, 2011  Resa et al., 2014a</td>
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<td>Natamycin</td>
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<td>Not GRAS / E235</td>
<td>Roller, 2003  Baines and Seal, 2012  Oliveira et al., 2007  Resa et al., 2014b  Fajardo et al., 2010</td>
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<td>Films</td>
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<td>Reuterin</td>
<td>Cheese Sausages Salmon Cottage cheese</td>
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<td>Langa et al., 2013 Gómez-Torres et al., 2014 Kuleaşan and Çakmakçi, 2002 Montiel et al., 2014 Lacroix, 2011</td>
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<td>Poly-L-Lysine</td>
<td>Sushi Rice Noodles Vegetables Soups Salads Steamed cakes Custard creams</td>
<td>Not GRAS / No E number</td>
<td>Baines and Seal, 2012</td>
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<tr>
<td>Lactoperoxidase</td>
<td>Milk Fruit juices Coatings</td>
<td>Not GRAS / No E number</td>
<td>Batt and Tortello, 2014 Touch et al., 2004 Cissé et al., 2015</td>
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<td>Lactoferrin</td>
<td>Baby formulas Meat</td>
<td>Not GRAS / No E number</td>
<td>Naidu, 2002 Giteru et al., 2015 Kaewprachu et al., 2015</td>
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<td>Essential oils</td>
<td>Meat Fish Dairy products Vegetables Rice Fruit</td>
<td>Not GRAS / No E number</td>
<td>Burt, 2004</td>
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<td>Carotenoids</td>
<td>Sauces Marinades Spice blends Coatings Beverages Milk</td>
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<td>Anthocyanins</td>
<td>Soft drinks Confectionary Fruit preparations</td>
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<td>Hendry and Houghton, 1996 Baines and Seal, 2012</td>
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<td>Chlorophylls</td>
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<td>Mustard, Yoghurt, Baked goods, Dairy products, Ice creams, Salad dressings</td>
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<td>Jams, Gelatins, Baked goods, Dairy products, Non-carbonated drinks</td>
<td>Approved / E120</td>
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<td>Dobbs and Bell, 2010</td>
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<td>Not GRAS / E960</td>
<td>Brandle at al., 1998</td>
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