Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the microbiological risks associated with the consumption of fruits obtained from *Fragaria* spp. and *Rubus* spp.

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Abstract

The consumption and as a result, the world production of fruits obtained from *Fragaria* spp. (such as strawberries) or *Rubus* spp. (such as raspberries or blackberries) has significantly increased worldwide in recent years. Spain is one of the largest producers of fruits from these plants, such as strawberries and raspberries. It is therefore of great relevance to know the microbiological risks associated with the consumption of these fruits, as well as the possible preventive measures to avoid the occurrence of outbreaks derived from berries intake.

To the present, these fruits have caused outbreaks mainly due to the presence of viral agents (especially norovirus and hepatitis A virus) and parasites (especially *Cyclospora cayetanensis*), whereas in the case of bacterial pathogens it has only been documented one outbreak caused by E*scherichia coli* O157: H7.

Given the heterogeneity of potentially contaminating pathogens in these products, and the fact that they are usually eaten raw or minimally processed, it is difficult to establish disinfection protocols to ensure their safety. For this reason, in order to maintain food security it is essential to maximize the good hygienic practices in cultivation and harvesting, especially in fruits cultivated at ground level. It is also necessary to maintain a standardized and internationally accepted traceability system that allows a rapid identification of the causing agent when an outbreak caused by the consumption of these fruits occurs.

Key words

Microbiological risk, prevention, disinfection, foodborne outbreak, strawberries, raspberries, blackberries, norovirus, hepatitis A virus, *Cyclospora cayetanensis, Escherichia coli* O157:H7.

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Introduction

In recent years, the *per capita* consumption of fruits and vegetables in the developed countries has undergone a major increase (Lynch et al., 2009), largely due to the recommendations of nutrition experts who advise increasing the consumption of these foods. These recommendations, represented in nutritional pyramids establishing the consumption of five pieces of fruit and vegetable per day, stem from the inadequate intake of these foods in western countries (WHO, 2003). It is known that these foods play a fundamental role in the prevention of diseases very common to our times, namely cardiovascular diseases, metabolic diseases such as obesity or diabetes and various types of cancer (Johnson et al., 2004). The preventive effect is due both to the fibre input and to the numerous bioactive and antioxidant substances (Arancibia-Avila et al., 2012). This is why consumers see these foods as safe and healthy but are often unaware of the risks derived from the presence in the same of food pathogens.

In this way, fresh fruits and vegetables are becoming more and more involved in the appearance of foodborne outbreaks. In the United States, the proportion of foodborne outbreaks in which vegetables have been identified as the causative agent has gone from less than 1% in the seventies to more than 6% in the nineties (Sivapalasingam et al., 2004). In Australia, fresh fruits and vegetables caused 4% of all the foodborne outbreaks reported between 2001 and 2005 (Kirk et al., 2008).

This rise can be attributed to the combination of multiple factors. In addition to the afore-mentioned increase in the *per capita* consumption of fresh fruits and vegetables, there are other major factors which are often linked to the appearance of food poisoning caused by the consumption of fruits and vegetables. These causes include the use in some producer countries of contaminants (such as manure or waste water). Another significant factor is the increasing distance and duration of transport from the place of origin to the place of consumption, increasing the probability of contaminations and/or proliferation of the contaminating microorganisms during transportation. The fact should also be noted that these foods are usually eaten raw and that the washing processes employed prior to their consumption may not be an efficient measure for eliminating the presence of pathogenic microorganisms (Burnett and Beuchat, 2001). Also important is the fact that there is an increasing tendency towards the marketing of these products in pre-cut form or with physical treatments that eliminate or reduce the protection offered by the vegetable peel or rind. Cutting or breaking the peel or rind results in the exit of nutrients from within the vegetable or fruit which may subsequently enhance bacterial growth, making these products more perishable.

More specifically, from among the fruits and vegetables, the consumption of fruits obtained from *Fragaria* spp. (including strawberries) and *Rubus* spp. (including raspberries or blackberries) has in recent years increased considerably at world level, with the result that their global production has, according to the FAO *(Food and Agriculture Organization)*, in 2009 reached 311,959 t in the case of blueberries, 409,707 t in the case of cranberries, 486,889 t in the case of raspberries and 4,178,152 t in the case of strawberries. In Spain, strawberries are of particular significance, as according to the FAOstat, Spain (together with the United States and Egypt) is one of the leading global exporters of strawberries, likewise raspberries, for which Spain is also one of the main global producers (together with Serbia and Poland) (*Codex Alimentarius* Commission, 2011).

Therefore, the Executive Director of the Spanish Agency for Food Safety and Nutrition (AESAN) has asked the Scientific Committee to prepare a report on the microbiological risks associated with the consumption of this type of product.

Epidemiological factors favouring the appearance of outbreaks caused by vegetable products

In the food chain from the farm to the fork there are a number of factors which may intervene decisively in the microbiological safety of fresh fruits and vegetables. External factors such as the environment in which the plants are grown, geographical location, type of risk, species of indigenous fauna in contact with the fruits and vegetables or harvesting methods may play a major role in the type and quantity of microorganisms that are found on the external surface of vegetable products (Brackett, 1999). In addition, certain intrinsic parameters such as the nature of the plant epithelial tissue and its protective covering, the interior pH of the plant, and the presence of natural substances with a potential antimicrobial effect condition the quantity and diversity of endogenous microbiota (Burnett and Beuchat, 2001). Nevertheless, the points in the food chain which entail a greater risk of contamination from pathogens are the cultivation of fruits and vegetables, the initial processing and their final culinary preparation (Lynch et al., 2009).

Recent studies indicate that the connections between the pathogenic bacteria which contaminate the food and plant products may be more complicated than a simple passive transfer (Tyler and Triplett, 2008). Thus, although pathogenic bacteria are well-adapted to survival in the intestines of vertebrate animals, they are also able to survive and proliferate with some ease in certain types of plants. This is the case of pathogens such as *Salmonella*, which are able to persist on plant surfaces for several weeks (Lynch et al., 2009). Pathogenic bacteria, and some viruses, may colonise inside the plants after entering through the root (Hirneisen et al., 2012), and once inside they are not affected by washing or surface disinfection. Thus they are able to spread by capillary action to all parts of the plant. As regards contamination during the production stage, this may be caused by direct contamination from food handlers' hands if these have not been correctly washed.

A number of authors have indicated that the most significant risk in the epidemiology of foodborne outbreaks caused by plant products is due to poor storage temperatures (Brackett, 1999) (Burnett and Beuchat, 2001). This is particularly true in the case of products imported from third countries, in which transport requires long times due to distance. An inadequate temperature favours the deterioration of the product and the proliferation of pathogenic bacteria which are often not native to our geographical zone, hindering the diagnosis in the case of a foodborne outbreak (Beuchat, 1998). The proliferation of viruses and parasites is not critical as they are inert particles outside the host cells. Nevertheless, even in the case of said agents, third country production, at great geographical distances, hinders the diagnosis and establishment of the source in the event of foodborne outbreaks. This is due to the non-existence in certain countries of a reliable system of traceability and/or rapid alert system such as those existing in the European Union.

Another significant risk factor is an inefficient surface disinfection and/or washing process. As a general rule, washing fruits and vegetables significantly helps to reduce surface microbial contamination and therefore extend their useful life and enhance food safety. Nevertheless, it should be noted that washing only eliminates a portion of the existing microbiota (Burnett and Beuchat, 2001) and is not equally effective for all pathogens.

There is also a significant epidemiological factor typical of industrialised countries: the gradual ageing of the population. The average age of the population is gradually increasing, bringing with it a greater number of individuals with chronic diseases and immunodepression, that may suffer from food poisoning caused by agents that would not be pathogens for young healthy individuals (Beuchat, 1998).

Pathogens causing foodborne outbreaks associated with the consumption of fruits from *Fragaria* spp. and *Rubus* spp.

The fruits of *Fragaria* spp. and *Rubus* spp. have been identified as foods involved in the appearance of outbreaks of viral gastroenteritis and hepatitis A (Butot et al., 2009). They have also been associated with outbreaks caused by *E. coli* O157:H7 (CDC, 2006) and protozoa (Cáceres et al., 1998) (Ho et al., 2002). Recently in Europe there have been several foodborne outbreaks caused by norovirus through the consumption of frozen raspberries imported from Poland, affecting a total of more than 1,100 individuals (Butot et al., 2007). As can be seen in Table 1, known foodborne outbreaks caused by the consumption of strawberries and raspberries were mainly provoked by viral agents and protozoa.

Table 1. Recorded foodborne outbreaks caused by the consumption of fruits from *Fragaria* spp. and *Rubus* spp.

Affected food	Infectious agent	Country	Reference
Mixed fruit	Hepatitis A virus	Canada	(CFIA, 2012)*
Raspberries	Hepatitis A virus	United Kingdom	(Ramsay and Upton, 1989)
Strawberries	Hepatitis A virus	United States	(Niu et al., 1992)
Strawberries	Hepatitis A virus	United States	(Hutin, 1997)
Strawberries	Hepatitis A virus	United States	(Hutin et al., 1999)
Blueberries	Hepatitis A virus	New Zealand	(Calder et al., 2003)
Blackberries	Norovirus	Germany	(Fell et al., 2007)
Raspberries	Norovirus	Finland	(Sarvikivi et al., 2012)
Raspberries	Norovirus	Europe-several countries	(Butot et al., 2007)
Raspberries	Norovirus	Sweden	(Hjertqvist et al., 2006)
Raspberries	Norovirus	France	(Cotterelle et al., 2005)
Raspberries	Norovirus	Denmark	(Korsager et al., 2005)
Raspberries	Norovirus	Sweden	(Le Guyader et al., 2004)
Raspberries	Calicivirus	Finland	(Ponka et al., 1999)
Raspberries	*Cyclospora cayetanensis*	United States	(Herwaldt and Ackers, 1997)
Raspberries	*Cyclospora cayetanensis*	United States	(Herwaldt and Beach, 1999)
Raspberries	*Cyclospora cayetanensis*	United States	(Ho et al., 2002)
Raspberries	*Cyclospora cayetanensis*	United States	(Cáceres et al., 1998)
Strawberries	*Escherichia coli* O157:H7	United States	(CDC, 2006)

*The outbreak was not confirmed but the berries were withdrawn from the market due to contamination.

Although to date only one outbreak caused by bacterial pathogens as a consequence of the consumption of strawberries and raspberries is known, the risk of this occurring cannot be minimised, as the paths of contamination of these fruit by enteric virus and bacterial pathogens such as *Salmonella* or *Escherichia coli* O157:H7 are, in global terms, the same (Bialka and Demirci, 2008). Moreover experiments have shown that both *Salmonella* and *Escherichia coli* O157:H7 can survive on the surface of strawberries for at least seven days (Knudsen et al., 2001). Nevertheless, it should be noted that the survival of the hepatitis A virus on nonporous inert surfaces is up to 60 days and on porous surfaces up to 30 days (Abad et al., 1994). Although there is no data for its survival on unprocessed strawberries or other similar fruits, it is estimated to be much higher than that of the afore-mentioned bacteria, which may contribute to its greater incidence in outbreaks transmitted by these foods. The main reservoir of *Escherichia coli* O157:H7 is cattle (Doyle et al., 1997), therefore it constitutes a risk for those berries grown at ground level, to which it has access through fertilisation with untreated manure or contaminated irrigation water. In addition, the possibility of future intoxications caused by other bacterial species such as *Listeria monocytogenes* or *Bacillus cereus* cannot be dismissed. Although there are no records of outbreaks as a result of the intake of this type of fruit, outbreaks have been caused following the intake of other types of fresh vegetable (Beuchat, 1998).

In the case of viral contamination, in addition to that caused by contact with faeces carrying the virus, individuals vomiting may be a major source of contamination if adequate measures of hygiene are not taken. These measures not only include washing hands but also the surfaces and tools potentially contaminated by the gases caused by the vomit, as they contain high viral concentrations, particularly in the case of infection by norovirus (Widdowson et al., 2005). It should also be added that hygiene measures must remain in place permanently and not only during times of recognised infection as there are many cases of asymptomatic carriers. Even in the particular case of the hepatitis A virus, the excretion of the virus in faeces is at a maximum when the symptoms of the disease have still not made themselves known (Pintó et al., 2012). As can be seen in Table 1, the viruses that have most frequently been identified as the cause of outbreaks derived from the intake of strawberries and raspberries are the hepatitis A virus and norovirus. Unlike bacterial agents, enteric viruses cannot multiply inside or on vegetables, which can only serve as a vehicle of transmission (Beuchat, 1998). The disinfection processes commonly applied to strawberries and raspberries are usually inadequate for eliminating or inactivating enteric viruses (Butot et al., 2008). The fact that some of the viral outbreaks included in Table 1 have occurred as a consequence of consuming products which have been frozen for several months clearly indicates that freezing does not render these viruses inactive (Falkenhorst et al., 2005) (Butot et al., 2008). To the contrary, it appears that freezing, and the lower the temperature the better, is the best method for preserving the infectivity of naked viruses.

Protozoan contamination normally occurs through irrigation water or contact with faeces, whether in manure, human faeces or domestic animal faeces. The establishment of general regulations regarding these agents is complex, as the cycles of each parasite differ, and therefore the paths required to infect a person through the intake of strawberries or raspberries cannot easily be standardised. Usually, the protozoa reach the plant surface via surface waters used in irrigation or by contact with manure in those countries in which it is used as a fertiliser (Beuchat, 1998). Another major factor to be considered is the fact that as some the varieties of these fruits are grown in tropical climates, their consumption may expose us to diseases native to this climate (Nóbrega et al., 2009).

Prevention of outbreaks caused by fruits of *Fragaria* spp. and *Rubus* spp.

It is important to remember that none of the treatments currently used in the disinfection of the fruits is totally effective in eliminating all the pathogens listed in Table 1. This is because these treatments are principally designed for bacterial elimination and, on the whole, both viruses and parasites are more resistant than bacteria. Therefore, the most efficient measures for preventing the appearance of foodborne outbreaks, as a consequence of the consumption of this type of fruit, must be directed at preventing or minimising the risk that the pathogens causing the outbreaks contaminate these fruit all along the food chain from the farm to the fork.

Even so, it is important to note that certain disinfectants or disinfecting processes have some effect on these pathogens. In the case of bacterial pathogens, there is a greater variety of chemical agents that may be used for disinfection, including chlorinated and iodine compounds, organic acids or quaternary ammonium compounds. The use of these disinfectants can increase the efficiency of washing to reduce surface bacterial contamination up to 100 times (Beuchat, 1998). Nevertheless, even when disinfectants are used, the elimination of surface microbiota is not complete. Some pathogens are resistant to the disinfectants used (for example *Listeria monocytogenes* is far more resistant to chlorinated disinfectants than *Salmonella*), and moreover there are specific zones of the plant epidermis that have a hydrophobic morphological structure, which cannot be reached by the disinfectant (Burnett and Beuchat, 2001). This mainly occurs if the microbial agent is not on the plant surface, but on the interior of the tissues, which it reaches through breaks in the plant epidermis or through the root.

Disinfection with quaternary ammonium compounds or HCl at concentrations 1:100 is also considered effective in rendering the hepatitis A virus inactive (Fiore, 2004). The use of phenolic compounds at high concentrations is also considered to be a good option for the elimination of certain types of virus (Gulati et al., 2001). Nevertheless, this option must only be considered for the disinfection of instrument surfaces as it significantly alters the organoleptic characteristics of fresh fruit. Therefore, in spite of certain exceptions, on the whole the efficiency of classic disinfectants is poor in the elimination of viruses or protozoan oocysts (Beuchat, 1998). This means it is necessary to consider other options which are more efficient against the agents usually involved in the appearance of foodborne outbreaks due to the intake of this type of fruit.

Agents which have proved to be efficient against enteric viruses and oocysts of certain protozoa such as *Cryptosporidium parvum* or *Giardia* include ozone and chlorine dioxide, thanks to their powerful oxidising action (Peeters et al., 1989). For this reason, some authors recommend the use of ozonised water at concentrations of 2-3 ppm for the disinfection of strawberries and raspberries (Beuchat, 1998).

It has also been shown that irradiation is active against parasites (Loaharanu and Murrell, 1994), and for rendering enteric viruses inactive. Nevertheless, a high radiation dose is required to eliminate these enteric viruses and therefore it is not recommended for use in foods (Mallet et al., 1991).

Other authors have indicated alternatives including the use of physical methods, such as heat treatments or vacuum freeze-drying (Butot et al., 2009). These methods are particularly interesting for those fruits which are not consumed fresh or as ingredients in minimally processed foods, as they are significantly effective against bacterial, viral and protozoan contaminants. Although the viruses may remain infective for long periods of time (more than a month) in the exterior at room temperature, heating the food to temperatures of more than 85 ºC for one minute is effective in rendering inactive certain viruses, such as the hepatitis A virus (Fiore, 2004). Nevertheless, it should be noted that certain enteric viruses, such as the hepatitis A virus, are even resistant to drying (Abad et al., 1994). Therefore the use of drying processes for strawberries, raspberries and other fruits in order to render the viruses inactive is not guaranteed. With respect to the protozoan oocysts, it has been shown that those of *Cyclospora cayetanensis* cannot sporulate after being frozen at -18 ºC for 24 hours, or after being heated at 60 ºC for 1 hour (Sterling and Ortega, 1999). The oocysts of this protozoa are also highly sensitive to drying, such that the cell walls are unable to withstand more than 15 minutes in a dry atmosphere (Chacín-Bonilla and Barrios, 2011), making it highly unlikely that these oocysts can be transmitted by strawberries or raspberries or other dried or freeze-dried fruits.

Physical methods also exist. These include the use of high pressures, efficient against bacterial contaminants as well as viruses such as the hepatitis A virus (Fiore, 2004). Nevertheless this methodology, in addition to being costly, is useful for juices, jams or jellies but not for fresh fruits, as this process damages the tissues (Malinowska-Panczyk and Kolodziejska, 2010). Another alternative is the use of pulses of ultraviolet light (Bialka and Demirci, 2008), which shows levels of activity similar to those of the most efficient disinfectants, at least in the elimination of bacterial pathogens such as *Salmonella* or *Escherichia coli* O157:H7. This procedure has also proved efficient in the elimination of fungi on the surface of raspberries (Lagunas-Solar et al., 2006).

Therefore, considering that there is no single method of disinfection which is efficient against all types of potential pathogens, prophylactic measures directed at preventing the contamination of fruits obtained from *Fragaria* spp. and *Rubus* spp. must be considered crucial for preventing foodborne outbreaks caused by these fruits. Therefore factors relating to the growth of the plants, their processing, transport and culinary preparation have a significant role. The following aspects are of particular relevance:

1. Water quality. The water used in irrigation as a medium for the application of treatments to the plants, or for washing the products after harvest, may be a major source of bacterial, viral or protozoan contamination. Similarly, the irrigation system used is very important, as unlike overhead irrigation, which moistens all of the plant, including the fruits, drip irrigation prevents the irrigating water from coming into contact with the fruit.
2. Isolation from faecal material. It is important to isolate fruits and vegetables from manure, domestic and wild animal faeces and human faeces. To ensure this, it is essential, especially in those plants whose fruits grow close to the ground, to establish physical barriers, for example plastic, that prevent contact with the same. If there is a risk that the fruits may have been in contact with faecal material, it is essential that these are not used chopped, peeled, cut or as an ingredient in minimally processed food, as the risk to consumers would be significant.

3. Correct washing and disinfecting processes. Both washing and disinfecting fruits and vegetables helps to reduce their surface microbial load. Nevertheless, even in the case of bacterial pathogens, these preventive measures are not totally efficient in the elimination of the same, especially if they are on the interior of the products. It must also be remembered that as mentioned above, protozoa and viruses are usually resistant to the disinfectants used. Therefore disinfecting these fruits only helps to reduce surface contamination. It is also important to consider that the surface of strawberries, raspberries, blackberries, etc., is not smooth, and there are hydrophobic morphological structures on the surface which hinder the entrance of the washing water and disinfectants used.
4. Storage at correct temperatures. Storage at mild temperatures suited to the specific plant species reduces the deterioration rate of the vegetables and the proliferation of the possible contaminating bacteria within. Special precautions must be adopted for those sliced or peeled products in which the external vegetable covering has been modified, as this favours bacterial contamination. On the whole, sliced or peeled products should be transported and stored in refrigerated chambers.
5. Good hygiene practices by food handlers. Correct hygiene, particularly with respect to the food handlers' hands, is crucial for reducing the appearance of foodborne outbreaks. In addition the existence of asymptomatic carriers of *Salmonella*, norovirus or the hepatitis A virus must be remembered. In fact, in the latter case the excretion of the virus in the faeces is at a maximum before the symptoms of the disease become apparent. This means that measures of hygiene should be maintained permanently and not only during recognised stages of infection.
6. Prevent the access of animals and insects to the above fruits, particularly those which have been washed and/or disinfected. Therefore, it is very important to use insulating packaging during marketing and to take special care during production, in the cases of fruits such as the strawberry which are grown at ground level.

Conclusions of the Scientific Committee

Given the epidemiological circumstances and the special features of the production of fruits of *Fragaria* spp. and *Rubus* spp., many of which are produced in countries which in some cases have a high prevalence of viral or parasitic disease, it is likely that the number of outbreaks caused by these fruits will continue to rise in forthcoming years unless specific measures for their prevention are adopted.

These fruits, due to the high activity level of the water and their rough surfaces, are susceptible to the proliferation of pathogenic bacteria. Therefore suitable washing and disinfection is recommended. Given the high survival rate of enteric viruses and parasites that have resulted in outbreaks reportedly caused by the consumption of these fruits, it is advisable to combine the washing and disinfection process with treatments which are able to reduce the virus and protozoa load. These include washing with ozonised water or with chlorine dioxide. Other interesting alternatives include the irradiation of those fruits from zones in which the presence of norovirus or the hepatitis A virus are more common, or the freezing of fruit from countries in which the presence of *Cyclospora cayetanensis* is enzootic.

Given the difficulties encountered in eliminating viruses and protozoa from the surface of the fruits obtained from *Fragaria* spp. and *Rubus* spp., it is appropriate to stress the need for good hygiene practices during production in order to prevent contamination of the same. In this respect, it is particularly important to prevent contact with faeces or untreated manure, the use of physical barriers to separate the fruit from the ground, the use of drinking water in irrigation and washing and the use of drip irrigation systems to prevent irrigation water coming into contact with the fruit. In the case of tools and surfaces in contact with the fruits, the use of phenolic compounds at high concentrations and the use of ultraviolet pulses are of interest.

It is essential to design a documented system of traceability, if possible accepted internationally, making it possible to rapidly determine the cause in those cases in which there is a foodborne outbreak, and to adopt suitable corrective measures. In addition it is vitally important to have rapid reliable methods for identifying the principal agents involved in the appearance of foodborne outbreaks as a consequence of consuming these fruits, together with methods of diagnosis that permit a fast and appropriate response in the event of an outbreak.

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