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Review article

Huitlacoche (corn smut), caused by the phytopathogenic fungus *Ustilago maydis*, as a functional food

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ABSTRACT

Background: In recent years the need has arisen to study and develop (or re-discover) foods that have nutritional characteristics as well as specific functions, such as improving health and/or reducing the risk of disease. For this reason knowledge of the nutritional value of food is important to promote greater consumer acceptance. In Mexico *huitlacoche* (also, *cuitlacoche*) has traditionally been prized as a delicacy since the time of the Aztecs and is currently being studied as a potential functional food and as a producer of natural bioactive substances that are used in fortifying foods.
Aims: To present an updated review about the properties of the *huitlacoche* (corn smut) as functional food.
Methods: A bibliographic search was performed and data were discussed.
Results: The data of the works reviewed here show that *huitlacoche* contains many compounds that confer to it unique organoleptic and nutraceutical characteristics.
Conclusions: The content of bioactive substances in *huitlacoche* supports the proposal that this is a good functional food as well as producer of compounds to enrich other foods.

El *huitlacoche* (tizón del maíz), causado por el hongo fitopatógeno *Ustilago maydis*, como alimento funcional

Palabras clave:
Ustilago maydis
Hongo fitopatógeno
Huitlacoche
Tizón del maíz
Alimento funcional

RESUMEN

Antecedentes: Durante los últimos años ha surgido la necesidad de elaborar, estudiar o redescubrir los alimentos que, además de proporcionarnos los beneficios nutricionales que los caracterizan, puedan cumplir una función específica, como mejorar la salud o reducir el riesgo de contraer enfermedades. Por este motivo, el conocimiento del valor nutrimental de los alimentos es importante para que estos tengan una mejor aceptación entre los consumidores. En México, el *huitlacoche* o *cuitlacoche* ha sido tradicionalmente apreciado como una delicia culinaria desde la época de los aztecas, y actualmente se está estudiando su potencial como alimento funcional y como productor de sustancias bioactivas naturales, que puedan ser utilizadas en la producción de alimentos fortificados.
Objetivos: Presentar una revisión actualizada acerca de las propiedades del *huitlacoche* (carbón del maíz) como alimento funcional.
Métodos: Se realizó una investigación bibliográfica y los datos fueron discutidos.
Resultados: Los datos de los trabajos revisados aquí indican que el *huitlacoche* contiene muchos compuestos que le confieren características organolépticas y nutracéuticas únicas.
Conclusiones: El contenido de sustancias bioactivas en el *huitlacoche* apoya la propuesta de que éste es un buen alimento funcional, además de producir compuestos para enriquecer otros alimentos.

The dimorphic fungus known as *Ustilago maydis* is responsible for the formation of corn smut, characterized by the formation of galls or tumors principally in ears, but also in stems and leaves of the plant host *(Zea mays)*[4] (fig. 1). This disease is usually considered a world-wide disease; nevertheless it has been used as food in

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Figure 1. Characteristics of *huitlacoche*, galls on corn cob induced by the phytopathogenic fungus *U. maydis.*

Mexico since pre-Columbian cultures[24]. As a result its popular name, *huitlacoche* or *cuitlacoche* comes from the word Nahuatl (the language of the Mexicas or Aztecs) "*cuitlacochin*" or "*cuitlacuchtli*" that means "degenerate corn on the cob". Also the significance of the word "*cuitlatl*" is "excrement", and that of "*cochi*" is "black" or "dark". In folk tales there are other interpretations of this term, including "crow filth" and "sleeping filth". In Nahuatl, this disease was also designated "*popoyauh*" or "*popoyotl*", meaning "burnt corn". For the Mexican excrement was not only a waste product in the physical sense, it also had a spiritual sense in which it was considered a distillation of food, which explains the root of the word. Indeed, "*cuitlatl*" comes from the name of a Mexica (Aztec) emperor named *Cuitlahuac*[36,39].

The use of *huitlacoche* as food has spread to the point that it is currently a culinary delight of international chefs due to the unique mixture of components that produce its flavor, aroma, and organoleptic characteristics[20]. Determination of total proteins, amino acids, dietary fiber, carbohydrates and unsaturated fatty acids have been made through nutritional studies, identifying *huitlacoche* as a functional food that produces bioactive substances, the latter of which can be used to create fortified food products. *Huitlacoche* is currently available in Mexico during the months of July and August, but in order to be marketed internationally it must be able to be produced year round, which requires the development of forms of production that can result in an economically feasible and nutritionally functional product of agreeable aroma, color and flavor.

Composition of free amino acids, volatile compounds and fatty acids, and their effect on the generation of flavor

Through derivatization studies, it has been determined that *huitlacoche* contains almost all the essential and many non-essential amino acids. The most abundant amino acid is lysine, corresponding to 14.84% of the total amino acids, followed by leucine, glycine, and aspartic acid (collectively representing 29.97% of the total amino acids in the sample (table 1 table 1)[21]. Also *huitlacoche* contains three of the four amino acids, which have an important effect in producing the umami flavor (that detected by the tongue principally because of the presence of glutamic, aspartic, tricholomic and ibotenic acid), resulting from their function as precursors or direct participants in the generation of this. Only ibotenic acid is missing from *huitlacoche*[21].

Table 1
Content of principal amino acids in *huitlacoche* and corn, % of total amino acids

Amino acid	*Huitlacoche*[21]	Corn[38]
Lysine	14.8	2.6-3.5
Leucine	10.4	12.0-15.8
Glycine	11.3	2.6-4.7
Aspartic acid	8.2	5.8-7.2
Methionine	0.69	1.98

Aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of static head-space analysis (GCO-SH) was used to identify the principal volatile compounds that confer the characteristic aroma of *huitlacoche*. Such compounds are hexanal, octanal, decanal, (*E,E*)-nona-2,4-dienal, (*E*)-undeca-2-enal, vanillin and sotolon[20]. The fact that the aromatic compounds of *huitlacoche* are principally aldehydes could owe itself to the oxidation of fatty acids, such as oleic or linoleic acid, which are the principal fatty acids of this food, constituting 40 and 30% of the total fatty acids, respectively (table 2 table 2)[20,45]. Linolenic and linoleic acids conforms one of the sources of two essential fatty acids, omega-3 and omega-6, respectively[9]. The high content of essential fatty acids suggests a high nutritional value for *huitlacoche*, and could be due to the fact that corn is one of the cereals with the greatest content of fats and with a good proportion of the essential unsaturated fatty acids as well (table 2)[38].

Content of mono and polysaccharides

Another factor that affects the organoleptic characteristics of a food product is the content of carbohydrates. In a study conducted by Valdez-Morales et al[44], 8 monosaccharides and 8 alditols were identified in samples of *huitlacoche*, of which two monosacharides, glucose and fructose, were the most abundant, together constituting approximately 81% of the total carbohydrates. Galactose, xilose, arabinose and manose were found in lesser proportions (table 3 table 3). Glycerol, glucitol and mannitol were the most representative of the alditols observed in the samples of *huitlacoche*[22].

The high content of glucose and fructose in *huitlacoche*, even more than the extra-sweet varieties of corn (table 3), suggests that a good proportion of this monosaccharide is a result of the infection of the fungus[44]. This idea is supported by the recent description of a novel high-affinity sucrose transporter *(srt1)* of *U. maydis*, which is expressed only during the infection of the host and its main role is the direct utilization of sucrose, that is in high levels in maize (table 3), without prior extracellular hydrolysis into

Table 2
Content of fatty acids in *huitlacoche* and corn, % of total fatty acids

Fatty acid	*Huitlacoche*[46]	Corn[38]
Oleic, 18:1	41-46	20.0-46.0
Linoleic, 18:2	27-34	35.0-70.0
Linolenic, 18:3	1.2-1.8	0.8-2.0
Palmitic, 16	14-18	7.0-19.0
Arachidic acid, 20	2.4-4.0	0.1-2.0

Table 3
Content of monosaccharides in *huitlacoche* and corn, mg/g of dry weight

Monosaccharide	*Huitlacoche*[44]	Corn[37]
Glucose	140-180	0.1-8-9
Fructose	60-100	Until 7.6
Glycerol	8.62	n.d.
Mannitol	3.17	n.d.
Sorbitol	4.45	Until 3.7
Sucrose	n.f.	4.4-94.6

n.d.: not determined; n.f.: not found.

monosaccharides[37,47]. The same could be true for the production of glycerol and mannitol. On the other hand, disaccharides like trehalose and sucrose were not detected in *huitlacoche* (table 3)[22,44].

Regarding the content of polysaccharides *huitlacoche* contains, as do other edible mushrooms, either homoglycans and heteroglycans which are part of the dietary fiber of a food[32,44]. Β-glucans are mushroom polysaccharides with a variety of structures, water-soluble or insoluble and that possess antitumor and immunostimulating properties. β-(1→3) backbone and β-(1→6) branch points are the most known antitumor structures[49]. β-glucans activates the complement and improve the response of the macrophages and killer cells. They can also be anti-oncogenic due to their protector effect against genotoxic compounds and because of their anti-angiogenic effect[1,32]. The content of β-glucans in *huitlacoche* is higher (between 1.0 to 12.0% of dry weight)[44] than that reported in corn (0.05-0.038%)[31,48] and similar to other edible mushrooms (2.45-11.10%)[23] (table 4 table 4). Among different maize genotypes tested to produce *huitlacoche*, corina cajete and biznaga creole, two types of creole corn, showed a high content of β-glucans and are proposed for the production of *huitlacoche* rich in β-glucans[44]. The content of total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are higher in *huitlacoche* than in corn (table 4), despite the fact that for example TDF in *huitlacoche* significantly decreases from 52% in the raw product to 49% when cooked[44]. The content of those dietary fibers in other edible mushrooms as *Boletus* spp. (commercial mixture), *Agrocybe aegerita* and *Pleurotus eryngii*[23] tend to be lower than in *huitlacoche* (table 4).

Polyphenolic compounds

Polyphenols are compounds characteristic of plants that have more than one phenol group per molecule[11]. Quercetine and kaempferol are polyphenols with low molecular weight that promise to be effective anti-cancer agents because of scavenging reactive oxygen species (ROS) and increasing the anti-cancer activity of drugs like dacarbazine (DTIC), ascorbic acid and N-acetylcystein (NAC)[5,43].

Although it has not been demonstrated that *huitlacoche* contains these compounds, the causal agent, *U. maydis*, produces enzymes like tyrosinase (E.C. 1.14.18.1) and laccase (E.C.1.10. 3.2) that catalyze the polymerization of hydroxy and mono phenols. While these enzymes were initially used in bioremediation, they have been utilized in the production of aggregates of relatively low molecular weight of quercetine and kaempferol, which have an anti-oxidant capacity greater than that of their monomeric precursors. It has been found that low concentrations of the systems of laccase-quercetine or laccase-kaempferol and tyrosinase-kaempferol have a strong effect on the inhibition of ROS and the lipoperoxidation of the membranes of a cell line of hepatocytes[7].

In addition, a symptom of the infection of corn by *U. maydis* is the production of anthocyanins[3], that are natural pigments characteristic of plants and are used as colorings and antioxidants in foods and pharmaceutical preparations, which are also polyphenolic compounds[30].

Indolic compounds

Indoles such as ascorbigen are found principally in foods like cabbage, broccoli and other crucifers. Experimental evidence suggests that these compounds confer a protector effect against cancer, especially breast cancer, since they inhibit estrogen type receptors[34].

Although the route of biosynthesis of indolic pigments and of production of auxines, such as indole acetic acid (IAA), has been described in *U. maydis*[35,50] it is still unknown if these elements confer some functional and/or organoleptic characteristics to *huitlacoche*. Nevertheless, the production of IAA in *huitlacoche* is important for the formation of the plant tumor that is part of the infection[35].

Protease production

Currently the proteolytic system of *U. maydis* is partially known[26], although in the study of proteases greater attention has been given to its function in relation to cellular physiology, these enzymes also favor the production of functional metabolites in foods and are involved in the generation of flavor[2]. It is known that various foods are source of peptides, which are inactive within the sequence of the native protein, but upon being liberated by enzymatic hydrolysis (in the intestine or in the process of production), can have beneficial biological effects on the organism. For instance, they act as antioxidants of lipoproteins and as regulators of biological processes[17,29].

In the case of *U. maydis*, four proteolytic activities have been detected (table 5 table 5) in the FB1 and FB2 haploid strains. The pumAe (extracellular), pumAi (intracellular) proteinases and the aminopeptidase pumAPE that have been purified and biochemically characterized[25,27,28] moreover pumAi has been related to dimorphic transition[28]. The characteristics of dipeptidyl aminopeptidase (pumDAP) were determined from a recombinant enzyme obtained by the heterologous expression of gen *dap2* of *U. maydis* in *Pichia pastoris*[14].

In many cases proteases of microbial origin, like neutral and alkaline proteinases, papain, and others, are added to food to improve the quality of its hydrolyzed products[2]. The proteases described in *U. maydis* could participate in the generation of these peptides in *huitlacoche* or contribute in some way to increase its nutritional value. Lysine is a limiting factor in the nutritional value of corn, but *huitlacoche* is one of the foods with the greatest content

Table 4
Content of dietary fiber and β-glucans in *huitlacoche*, corn and other edible mushrooms, % of dry weight

Polysaccharide	Huitlacoche[44]	Corn	Boletus spp[23]. (commercial mixture)	Agrocybe aegerita[23]	Pleurotus eryngii[23]
TDF	40.0-60.0	7.0[31]-12.9[48]	4.27-12.09	5.07-7.39	4.64-6.65
SDF	10.0-30.0	1.5[48]	0.58-2.38	0.96-1.37	0.53-0.80
IDF	30.0-50.0	5.0-10.0	3.69-10.18	4.11-6.02	4.11-5.84
β- glucans	1.0-12.0	0.05[48]-0.38[31]	2.45-11.10	3.02-3.67	4.13-5.16

IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF: total dietary fiber.

Table 5
Biochemical characteristics of the *U. maydis* proteases

Protease	Protease classification	Mr (kDa)	p*I*	Substrate
Proteinase pumAe[25] (extracellular)	No aspartyl protease	72-74	5.5	Hemoglobin
Proteinase pumAi[27] (intracellular)	Aspartyl-protease	35.3-36.6	5.5	Hemoglobin
Proteinase pumBe (extracellular)	n.d.	n.d.	n.d.	Hide Powder Azure (collagen)
Proteinase pumBi (intracellular)	n.d.	n.d.	n.d.	Hide Powder Azure (collagen)
Aminopeptidase[28] pumAPEi (intracellular)	Metallo-protease	58	5.1	Lys-pNA
Dipeptidyl[14] aminopeptidase pumDAPi (intracellular)	Serine-protease	123	6.9	Ala-Pro-pNA

n.d.: not determined.

of this amino acid. Of course, all sources of nutritional proteins tend to supply functional peptides[17].

With the availability of the *U. maydis* genome[15] the search for and study of codifying genes of other proteases is relatively easy. Given the relevancy which is now recognized regarding the consumption of foods that contain compounds with antioxidant activity, the identification of proteolytic enzymes that generate these products is becoming very important.

Biosynthesis of glycolipids and biosurfactants

Biosurfactants, which are biological molecules with surfactant or tensoactive properties produced on surfaces of microorganisms or secreted by the same, have various advantages over chemical surfactants, including low toxicity, high biodegradability, environmental compatibility, selectivity and specific activity at high temperatures, as well as extreme pH and salinity[16].

In conditions of nitrogen starvation, *U. maydis* produces great quantities of two biosurfactants, derived from two classes of glycolipids: ustiliagic or celobiosid lipid acid (UA) and ustilipid or lipid mannosylerythritol lipid (MEL)[12,18,19,42]. The secretion of UA is critical in the antagonic effect of *U. maydis* on *Botrytis cinerea* when they are co-inoculated on tomato leaves. In the same sense, MEL from *Candida antartica* also presents antimicrobial activity[42]. Accordingly, the use of biosurfactants as agents of biocontrol could be a new field of action for these metabolites.

Huitlacoche production

Huitlacoche is sold raw, in prepared food or processed in Mexican local markets during July and August (the second half of the rainy season), reaching between 400 and 500 tons of product per year[10]. However the introduction of this food into the international market, in countries as US, Japan, China and some of the European Community, as France, Spain and Germany for example[13], requires the development of techniques that will allow for the production of large quantities during the whole year.

The factors that can affect the production of *huitlacoche* are the efficient production of the inoculant, the timing of inoculation and harvest, the characteristics of the corn hybrid which are used, and the strains of *U. maydis* utilized[33,46]. For instance, the taste, aroma and nutritional value of *huitlacoche* are factors that are dependent on the variety of corn and the state of development in which the fungus is harvested[44].

For production of *huitlacoche*, the inoculant must be obtained in a controlled process. First, two sexually compatible strains of *U. maydis* are kept separate in a medium of acidic potato dextrose agar (aPDA) and cultivated separately in 50 mL of potato dextrose broth (PDB), under constant stirring at room temperature for 18 to 24 h, to obtain the preinoculant. Then 0.5 mL of this preinoculant is cultivated in 100 mL of PDB under constant stirring for 12 to 18 h in order to obtain the inoculant. For plant inoculation, the basidiospores of each strain are adjusted to a concentration of 10^6 cells/mL of medium and then mixed together[33].

In a report about the production of biomass by the Taguchi method, the pH of the medium was determined to be 7, the velocity of stirring 200 rpm, and the glucose concentration in the medium 40 g/L. These are the most important factors in the production of the biomass of the FBD12 *U. maydis* strain. By using the before mentioned conditions, a maximum biomass of 15.67 g/L was obtained in 48 h[6].

The inoculation of the mixture of sexually compatible strains in the corn plants is administered by means of an injection of the suspension of spores in the silk-channels of the corn or in the leaves once the silk has emerged[40]. Sweet corn hybrid varieties are the most susceptible to infection. Field studies suggest that the infection is more severe when the plants are inoculated between 4-8 days after the mid-silk growth state[33], which can be tested by pressing a grain of corn with a fingernail to see if a milky substance comes out. Maximum yields of *huitlacoche* and the best quality product (including color and flavor) are obtained 16 to 17 days post inoculation[33,45]. Pollination will reduce the formation of galls up to 50%[8,41]. In spite of the fact that the control of polinization can be costly, it is necessary for quality control of the commercial production of *huitlacoche*.

Taking all of these factors into account can make the commercial production of *huitlacoche* functional, thus offering the international market with a food of fine organoleptic qualities year round.

Conflict of interest

None declared.

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