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REVIEW

A review on inactivation methods of *Toxoplasma gondii* in foods

Adel Mirza Alizadeh[a], Sahar Jazaeri[b], Bahar Shemshadi[c], Fataneh Hashempour-Baltork[a], Zahra Sarlak[a], Zahra Pilevar[a] and Hedayat Hosseini[b]

[a]Student Research Committee, Department of Food Technology, Faculty of Nutrition Sciences and Food Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [b]Department of Food Science and Technology, Faculty of Nutrition Science, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [c]Department of Parasitology, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran

ABSTRACT

Toxoplasmosis is an infection caused by *Toxoplasma gondii*, a widespread zoonotic protozoan which poses a great threat to human health and economic well-being worldwide. It is usually acquired by ingestion of water contaminated with oocysts from the feces of infected cats or by the ingestion of raw or undercooked foodstuff containing tissue cysts. The oocyst can contaminate irrigation water and fresh edible produce. It is estimated that approximately one-third of the human population worldwide harbor this parasite. Infection with *T. gondii* is an important cause of diseases of the central nervous system and the eye in immunocompromised and immunocompetent individuals. The purpose of this study was to evaluate the efficacy and applicability of thermal (heating, cooking, freezing and low temperature), non-thermal (high pressure processing, ionizing irradiation and curing) and chemical and biochemical (disinfection, essential oils and biochemical methods such as enzymes, nanoparticles, antibiotics and immune response) treatments for the inactivation, inhabitation or to kill *T. gondii* in foodstuff intended for public consumption and under experimental conditions.

KEYWORDS

Toxoplasma gondii; foodborne toxoplasmosis; inactivation treatment; thermal; non-thermal; chemical and biochemical

1. Introduction

Foodborne diseases are caused by a number of agents that can affect or compromise the life of the consumer and in most cases are of biological origin, such as bacteria, viruses and parasites. Food and waterborne infections have received considerable attention for many years. Parasites are organisms that acquire nourishment and protection from other living organisms such as humans and animals that are known as hosts. They can cause illness in humans when present in food or water. Many parasites can be transmitted by food and food handlers, including protozoa and helminths.

In many countries, the most common foodborne parasites are protozoa such as *Cryptosporidium* spp., *Entamoeba histolytica, Cyclospora cayetanensis, Giardia intestinalis, Sarcocystis (hominis and suihominis), Toxoplasma gondii*, roundworms such as *Anisakis* spp. and *Trichinella* spp., flatworms such as *Fasciola hepatica, Fasciolopsis buski* and *Paragonimus sppand* tapeworms such as *Diphyllobothrium* spp., *Taenia* spp. and *Echinococcus* spp. One common zoonotic parasitic disease worldwide is toxoplasmosis, an infection caused by *T. gondii* [1].

T. gondii cannot grow outside of a suitable host, in all food types or in other environments; however, findings have shown that *T. gondii* infection can be transmitted by the ingestion of oocysts (from contamination of the environment through cat feces) and can contaminate drinking or surface water, soil (an oocyst can survive in soil for up to two years) [2] and fruits and vegetables or by viable tissue cysts found in raw or undercooked meat of intermediate hosts (all warm-blooded animals, including most livestock and humans) [3,4]. The oocysts are highly infectious to herbivores, as are the bradyzoites to cats. There are three infectious stages of *T. gondii*: in groups or clones as a tachyzoites, in tissue cysts as a bradyzoites and in oocysts as sporozoites. Biological life cycle of *T. gondii* is classified in the sexual and asexual stages. The sexual cycle is restricted to the feline intestine and lead to shedding of oocysts in cat feces. The activated oocysts (excretion by cat) become extremely infectious and can survive in the environment for long time (several months) and possibly years. The asexual cycle is initiated when any other warm-blooded animal ingests these infectious oocysts [5].

Food handlers such as farmers, sellers, butchers or housewives that directly or indirectly deal with the production, preparation, processing and distribution of foods between communities are identified in most cases as the most likely sources of toxoplasmosis infection in humans. One of the best ways to prevent

CONTACT Bahar Shemshadi bshemshadi@yahoo.com Department of Parasitology, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran

contamination is compliance with good hygiene practices during food production and processing [6].

In response to natural infection, most farm animals are seropositive for *T. gondii*. Serological studies have shown infectious parasites of *T. gondii* in meat production of farm animals [7]. Food animals such as pigs (extreme seroprevalence in many countries) [8,9], poultry (seroprevalence up to 65% in free-range chickens and 81% seropositive in birds) [10] and sheep and goats (seroprevalence of 75 to 92% in many areas of the world) [11] become infected by the same routes, resulting in meat products containing tissue cysts which can then infect consumers [12]. Scientists have reported that at least a third of the global population is infected with the parasite, making it one of the most successful parasitic infections [13].

Researchers have indicated that although infection with *T. gondii* in healthy humans may be asymptomatic, it can be cause substantial risks and be fatal to immunocompromised individuals such as young children, the elderly, HIV/AIDS and cancer patients and organ transplant recipients [14–18]. In addition to consumption of contaminated meat or infection with feline feces, it can cause trans-placental infection in pregnant women when the infection is acquired during pregnancy [19]. Studies have shown a 30 to 90% toxoplasmosis infection rate in Central and South America and continental Europe [20–22]. The estimated disease burden (incidence, mortality and sequelae) of congenital toxoplasmosis in the Netherlands was 620 (range of 220–1900) disability-adjusted life years (DALY) [23], which is similar to that reported in the US [24]. High burdens have been reported in South America and some Middle Eastern and low-income countries as well [25]. Hoffmann et al. reported that the annual cost of illness and quality-adjusted life year loss (QALY) in the US is due to foodborne pathogens. They estimate that the cost of *T. gondii* is $3 billion annually (11,000 QALY) [24].

2. Impact of *T. gondii* on health and reproduction of farm animals

The development of the food chain supply, extension of international travel, and increase in the populations of vulnerable groups, changes in dietary habits and improved diagnostic methods and communication are factors that have given rise to the investigation of foodborne parasitic diseases globally [26]. In two advanced studies of death and disability attributable to pathogens of foodborne diseases, toxoplasmosis was classified as very high [27] and many researchers have presented similar findings in different parts of the world [24,28,29]. Economically, it causes tremendous loss of valuable livestock (cattle, pigs, sheep, goats and poultry).

Infection of dairy goats with *T. gondii* is widespread and constitutes a public health concern [30], resulting in significant reproductive loss [31,32]. Jacobs and Hartley reported that infection within 30 days after mating led to fetal death and resorption in 42% of ewes. When infected 90 days after mating, 16% of ewes aborted, and 16% gave birth to dead lambs and 52% to live lambs [33].

These results and risk assessment suggest that *T. gondii* infected animals are a food safety concern. In terms of food safety, the US Food and Drug Administration reports that about 85% of pregnant women in the US are at risk of being infected with toxoplasmosis. About 50% of toxoplasmosis infections in the US each year are acquired from food [34]. The consumption of lamb, beef and meat products such as salami are more common in northern and central European countries than in Italy. Cook et al. found that the proportion of toxoplasmosis infections attributed to eating salami was 14, 11, 4, 3, 10 and 5% in Naples, Milan, Copenhagen, Oslo, Brussels and Lausanne, respectively [35].

3. Food contamination

Contamination of food and the environment by *T. gondii* can directly or indirectly cause infections in humans consuming high risk food products such as contaminated meat (sheep, goats, pigs, cattle and birds), unpasteurized milk (unpasteurized or inadequately processed milk or fresh cheese), fresh or raw fruits, vegetables and plant products, as well as contaminated water [36]. Infection may also occur by consumption of raw or undercooked meat containing the cysts or exposure to cross-contamination through water and food contaminated with feline feces [19]. Cook et al. showed that 30 to 63% of infections in European countries could be attributed to raw and undercooked meat consumption [35]. Different types of foodstuff, such as pork [37], sheep [38], poultry [21] and cattle [39], the unpasteurized milk of goats [4], fresh plants and plant products [40], water [3] and raw or undercooked seafood [41] are food vehicles in the transmission of toxoplasmosis to humans (Figure 1).

As noted, toxoplasmosis is acquired by ingesting food and water contaminated with oocysts from the feces of infected cats. However, it can also occur by the ingestion of infected meat containing tissue cysts of an infected intermediary host [3]. In the US, assessment of 698 retail outlets showed that the prevalence of viable *T. gondii* tissue cysts in commercially available fresh pork products was 0.38% [42]. Investigation of 71 meat samples obtained from UK retail outlets showed the presence of this parasite in 27 of the meat samples [43]. Another study reported that *T. gondii* was detected in ready-to-eat cured meat samples [44]. Evaluation of the prevalence of *T. gondii* in meat and

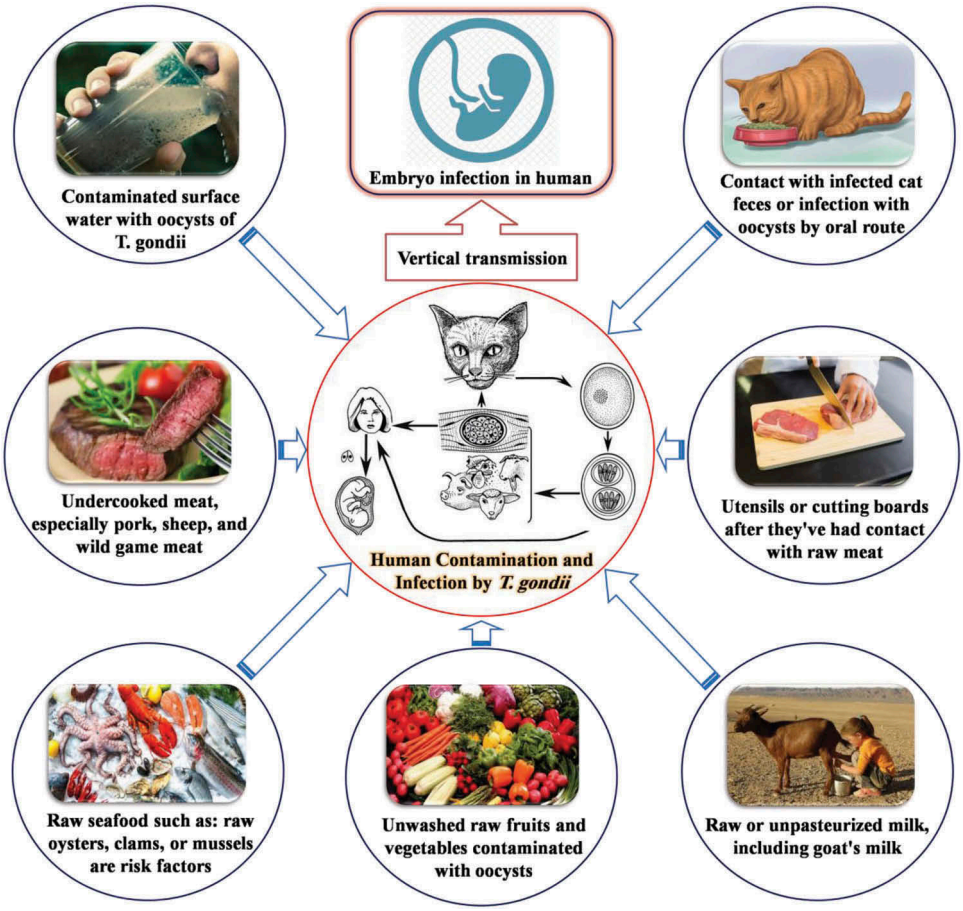

Figure 1. Main sources of *T. gondii* infection in food or by oral entry route in humans.in humans.

meat products (100 samples consisting of tongue, heart and muscle taken from 50 lamb and 50 beef samples) in Ahvaz, Iran showed that 7 lamb samples (14%) and 2 beef samples (4%) were found positive for *T. gondii* cysts [45].

Although outbreaks of toxoplasmosis infection in humans are recognized infrequently, outbreaks associated with water and food are shown in Table 1. The world's largest outbreak of waterborne toxoplasmosis occurred in British Columbia in Canada, for which drinking water was the source [46].

4. Inactivation treatments for *T. gondii*

Tachyzoites, bradyzoites and sporozoites are the three infectious stages of *T. gondii* in humans and other warm-blooded animals. Humans and animals become infected mainly by ingesting bradyzoites or oocysts [56]. *T. gondii* oocysts are highly resistant to environmental influence and are rapidly inactivated following exposure to temperature extremes, irradiation, chemical agents and other physical methods.

It has been found that the oocyst wall is bilayered, with the outer layer being thinner than the inner layer [57]. Experimental results have shown that layers are not tightly bound together and the outer layer can be stripped off easily using chemical agents [58]. Furthermore, the oocyst wall is composed of more than 90% cysteine- and tyrosine-rich protein [59] and thermal methods such as high heating, cooking or physical methods can denature the proteins and cause inactivation or killing of *T. gondii*. The use of drugs or freezing can inactivate or prevent *T. gondii* infection by preventing the parasite from attaching and entering cells [60] or by disrupting metabolism and the formation of ice crystals [61,62], respectively. Under experimental and industrial conditions, physical methods (thermal and non-thermal) or other applied technologies (chemical and biochemical methods) can be used to control, remove or inactivate *T. gondii*. The prevention of cross-contamination in raw and processed food products is significant in this regard (Figure 2).

5. Thermal methods

The killing or inactivation of the parasitic cysts in animal tissue and oocysts in the environment is essential to preventing *T. gondii* infection in man and animals. Studies have reported beneficial effects from heating, freezing and cooking for inactivation of *T. gondii* [63].

Table 1. Reported toxoplasmosis outbreaks associated with water and foods.

Years	Country	Type of food	Ref.
1992	France	Undercooked or cured meat products	[47]
1994	South Korea	Uncooked pork	[48]
1994–1995	Denmark-Belgium-Italy-Switzerland-Norway	Undercooked or cured meat products	[35]
2001	India	Contaminated water	[49]
2001–2002	Brazil	Contaminated water and/or foods	[50]
2001–2002	Brazil	Ice cream prepared with contaminated water	[50]
2004	India	Contaminated water	[51]
1999–2004	United States	Contaminated water	[52]
2005	Suriname	Contaminated water	[53]
2006–2008	Poland	Fruits and vegetables	[40]
2009	Brazil	Vegetable	[54]
2009–2010	United States	Contaminated water	[52]
2014	Tunisia	Unpasteurized goat milk	[55]

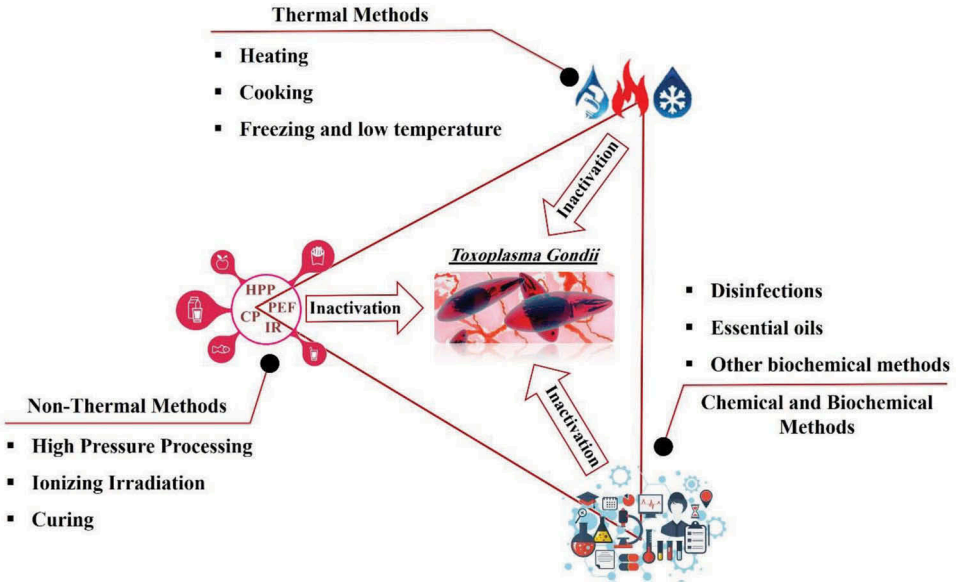

Figure 2. Schematic of applicable methods for inactivation of *T. gondii* in food.

5.1. *Heating*

Heat processing is an important method for extending the storage life of foodstuff. The basic purpose of heat treatment of food is to reduce or destroy all microbes or spoilage caused by bacteria and bacterial spores, viruses and parasites. Heat processing of food products for sterilization must be intense enough to inactivate or kill most heat-resistant microorganisms [64]. *T. gondii* is susceptible to heat inactivation and studies have shown that heating can destroy *T. gondii* oocysts of both unsporulated and sporulated strains [65].

Dubey et al. reported on the destructive effect of heating on tissue cysts [66]. Another study showed that heating at 58°C was sufficient to inactivate all oocysts [67]. Wainwright et al. indicated that *T. gondii* oocysts may not always be inactivated when exposed to a minimum of 60°C for 1 min and that the water heating time, cooling time and volume of water treated must be considered when evaluating radiofrequency (RF) or thermal heating for oocyst inactivation [68]. Table 2 shows that heating methods are effective for causing *T. gondii* to become inviable. Shorter times and lower temperatures are needed to kill tachyzoites found in the heart (a blood-filled organ) that recently have been acquired from the animal compared to bradyzoites, which could be found in muscle tissue and most other tissues. Long times/temperatures/treatments are needed for oocysts found in contaminated vegetables or in the gut tissue of felines.

5.2. *Cooking*

The primary control factor for prevention of *T. gondii* infection in meat consumption is adequate cooking and prevention of cross-contamination by separation of cooked meat and raw or undercooked meat [69]. Hill et al. showed that *T. gondii* is killed in 5.6 min at 49°C, in 44 sec at 55°C and in 6 sec at 61°C if the temperature is evenly distributed and maintained throughout the meat [70]. Other studies have reported similar findings (Table 2). Overall, meat should be cooked to 67°C before consumption and tasting meat while cooking or while seasoning should be avoided [71]. Briefly, cooking meat and its products

Table 2. Summary of effects of thermal methods to inactivation of *T. gondii* and its infectious forms.

Type of methods	Temperature(s) (°C)	Time (min/h/day)	Main finding	Ref.
Heating	50	2.5 min	Sporulation of *Toxoplasma* oocysts inhibited.	[65]
	50	30 min	Infectivity of sporulated oocysts disappears.	[65]
	55	30 min	Tissue cysts are destroyed.	[66]
	58	30 min	No evidence of parasites in infected murine brains.	[67]
	58	15 min	Sufficient to inactivate all oocysts.	[67]
	61	3.6 min	Tissue cysts were generally rendered nonviable.	[75]
	60 or 100	1 min	No viable *T. gondii* infective stages isolated from meat samples.	[76]
	63	30 min	*T. gondii* tachyzoites RH strain die in pasteurized milk.	[77]
	75	1 h	Heat treatment like boiling water can inactivate *T. gondii* oocysts.	[78]
Cooking	63, 71, 82	–	Beef, lamb and veal roasts and steaks should be cooked to at least 63°C. Pork, ground meat and wild game should be cooked to 71°C before eating. Whole poultry should be cooked to 82°C in the thigh to ensure doneness.	[27]
	67	–	Tissue cysts in meat are killed by heating meat throughout.	[61]
	50	1 h	Heating inactivates tissue cysts.	[73]
	67	0.01–96 min	Kills tissue cysts in meat.	[79]
	66	-	Heating meat throughout to reach a temperature is sufficient to kill cysts in meat.	[80]
Freezing and low temperature	−12.37	11.2 day	Nonviable *T. gondii* tissue cysts in pork upon freezing.	[61]
	−20	1–2 day	Tissue cysts stored at −20°C could infection after 24 and 48 h of storage.	[81]
	−21	1 1/2 h	After 1 1/2 h of exposure to −21°C, many cysts seem to lose their infectivity.	[62]
	−10 or −20	3 day	Freezing of meat at −10°C for 3 days or at −20°C for 2 days killed parasite and cysts could not recover.	[76]
	−7	4 day	Inactivation of T *gondii* tissue cysts was achieved by freezing at −7°C for 4 days.	[82]
	−20	21 day	Sporulated oocysts were inactivated by freezing.	[82]
	−20	2 day	Freezing for 2 days at −20°C was sufficient to inactivate parasite.	[72]
	−25	6–35 day	Experiments with meat from pigs fed with *T. gondii* infected mice showed that all meat samples were rendered non-infectious.	[83]
	−7–12	4 day	Parasites in meat from experimentally infected pigs did not survive.	[82]
	−20	3 day	Temperature and time required to inactivate isolated tissue cysts.	[81]

to reach the desired temperature throughout (60–70° C) is sufficient to kill *T. gondii* cysts.

5.3. *Freezing and low temperature*

Freezing of food by consumers is common in many countries, but an important factor in consumer attitudes towards freezing is the perceived loss of sensory qualities [7]. Studies have shown that freezing can inactivate the *T. gondii* tissue cysts. The effect of freezing on *T. gondii* cyst viability was first described in 1965 by Sommer et al., who stated that freezing for two days at −20°C was sufficient to inactivate the parasite [72]. Other studies have shown that to inactivate the isolated tissue cysts, at least three days at −20°C is required and these results are in accordance with earlier reports by other researchers [73,74]. Table 2 shows similar studies on the effect of low temperature and freezing on the killing or inactivation of *T. gondii* and its infectious forms. It has been recommended that meat be stored for at least three days at −20°C to reduce the *T. gondii* load in contaminated meat.

6. Non-thermal methods

In the last decade, non-thermal technologies have become of interest to food scientists, manufacturers and handlers because they have minimal impact on the sensory properties and nutritional properties of food. They also can extend the shelf life by inactivation or killing of foodborne pathogens [84,85]. Novel non-thermal technologies such as pulsed electric fields (PEF), pulsed light treatment (PLT), high pressure processing (HPP), cold plasma (CP) and extremely low frequency (ELF) and ionizing radiation (IR) have the ability to inactivate a range of bacteria, viruses and parasites [86,87]. This section summarizes some non-thermal processing technologies that are currently available or in development for the inactivation of *T. gondii*.

6.1. *High pressure processing*

HPP technology is a relatively new, non-thermal method that subjects food products (liquid or solid) to pressures of up to 1000 MPa to inactivate or kill

many of the microorganisms which are found in foods with rapid processing times, even at low temperatures, without losing minerals, vitamins, flavor and color molecules in the process [84,88,89]. HPP is also known as high hydrostatic pressure (HHP) or ultra high pressure processing. It has been shown to be effective for the elimination of microbial spores from a variety of food products [90]. Studies on the effectiveness of HPP in the killing or inactivation of foodborne parasites show the sensitivity of the parasites and achieves their destruction at relatively low pressures [7].

Lindsay et al. have shown that HPP using 340–550 MPa can inactivate *T. gondii* tissue cysts under laboratory conditions [91]. However, the effects of high pressure treatment on the color and texture of food have limited consumer acceptance [10]. Similar studies on HPP treatment of *T. gondii* are presented in Table 3. The results suggests that 340–400 MPa for 1 min is applicable to control or inactivate *T. gondii* in food, but further studies are needed to determine the amount of time and HPP needed for each *T. gondii* life cycle.

6.2. *Ionizing irradiation*

IR is a non-thermal food pasteurization process that reduces, inactivates or eliminates spoilage of pathogenic microorganisms, insects, fungi and other pests

Table 3. Summary of effects of non-thermal methods to inactivation of *T. gondii* and its infectious forms.

Type of methods	Experimental Conditions	Main finding	Ref.
High Pressure Processing	400 or 300 MPa to 30, 60, or 90 sec	No mice inoculated with tissue cysts exposed to 400 or 300 MPa became infected.	[104]
	340 MPa to 1 min	Use of HPP at 340 MPa for 60 sec required to render oocysts spot inoculated on raspberries non-infectious for mice.	[104]
	550 MPa, 480 MPa, 400 MPa, or 340 MPa to 1 min	*T. gondii* oocysts in HBSS (without calcium or magnesium) or distilled water treated with HPP at 550, 480, 400 or 340 MPa for 60 sec were rendered noninfectious for mice.	[105]
Ionizing Irradiation	Gamma (70 krad)	Use of 70 krad gamma radiation was minimum effective dose for fresh pork.	[93]
	Gamma (40 krad)	*T. gondii* in tissue cysts killed by exposure to 40 krad of gamma irradiation.	[93]
	Gamma (50 krad or more)	Tissue cysts irradiated with 40 krad were infectious when inoculated in mice, but when irradiated with 50 krad or more, tissue cysts were not detected.	[107]
	Gamma (70 krad or 100 krad)	Tissue cysts in murine brains and edible pig flesh irradiated with 30 and 50 krad doses were not effective, whereas irradiation with 70 or 100 krad did not infect cats or mice in bioassay.	[108]
	Gamma (20 krad)	Irradiation treatments at doses as low as 20 krad effectively inactivated *T. gondii* oocysts on blueberry surfaces with minimal impact on texture, color, or anthocyanin content of treated berries.	[109]
	Gamma (60 krad and 45 krad)	The minimal effective dose for Chinese NT strain and the American ME-49 and TS-2 strains of *T. gondii* cysts in mouse and pig tissues was 60 krad. The infectivity for mice of NT strain bradyzoites irradiated at 45 krad decreased 10,000-fold.	[95]
	Ultraviolet (>20 mJ/cm^2)	A 4-log inactivation of the oocyst/sporozoite infectivity was obtained for UV fluence.	[110]
	Ultraviolet (4 mJ/cm^2 and 10 mJ/cm^2)	The results from the animal bioassay show that 1- and 3-log10 inactivation was achieved with 4 mJ/cm^2 UV and 10 mJ/cm^2 low-pressure UV, respectively.	[94]
	Ultraviolet (40 mJ/cm^2)	A 2-log10 reduction of *T. gondii* oocyst infectivity was achieved at 40 mJ/cm^2.	[111,112]
	Ultraviolet (>500 mJ/cm^2)	Inactivation of *T. gondii* oocysts occurred with exposure to pulsed and continuous UV radiation, as evidenced by mouse bioassay. Even at >500 mJ/cm^2, some oocysts retained their viability.	[113]
	Ultraviolet (1 min UV exposure)	Using 1 min UV light at 3689.04 μJ/cm^2/sec powers for a total energy exposure, tachyzoites were unable to replicate in vitro or produce parasite cysts in vivo.	[96]
Curing	3.9% NaCl, 25 mg/kg nitrate, and 3 mg/kg nitrite; 14 months	The last curing salt concentration of 3.9% NaCl, 25 mg/kg nitrate and 3 mg/kg nitrite for a duration of curing of 14 months inactivated *T. gondii*.	[114]
	2.5% of sodium nitrite; 14 days	About 2.5% of initial amount of sodium nitrite was effective for killing *T. gondii* cysts in 14 days.	[115]
	7% nitrates, 4% nitrites, sodium ascorbate, and sodium chloride; 9–12 months	The viability of *T. gondii* was higher in hams cured for 9 months compared to those cured for 12 months.	[116]
	2.0% NaCl or 1.4% or higher lactate-based salt solutions; 8 h	The injection of 2.0% NaCl or 1.4% or higher lactate-based salt solutions into pork loins containing infective tissue cysts within 8 h prevented transmission of *T. gondii*.	[103]
	salt and sugar for 64 h at 4°C; smoking at 50°C to 24–28 h	Curing of lamb meat with salt and sugar for 64 h at 4°C or smoking salt-injected meat at temperatures not exceeding 50°C for 24 to 28 h was effective for killing *T. gondii*.	[110]
	6% NaCl; 4–20°C; 3–56 days	In various time intervals and all temperatures examined, tissue cysts were killed in 6% NaCl solution.	[101]
	2.0 and 2.5% of salt; 48 hours	Pig sausage experimentally inoculated with *T. gondii* showed that salt in concentrations of 2.0 and 2.5% inactivated the parasite within 48 h of onset of curing.	[117]
	3% table salt; 3–7 days	About 3% table salt after 3–7 days killed *T. gondii* tissue cysts.	[118]
	2.5 and 3.0%, NaCl and 0.5% nitrite; 1–8 day	The cysts lost their infectivity in concentrations of 2.5 and 3.0% NaCl after 1 day. NaCl plus 0.5% nitrite had a stronger effect on *T. gondii* cysts than common table salt.	[119]

and retards the spoilage of food. IR can use gamma rays from radioisotopic sources such as cobalt-60 or cesium-137, electrons, x-rays from beta rays, electron beam accelerators or ultraviolet rays (from an electromagnetic spectrum UV region) [85]. Irradiation has been evaluated for inactivation or killing of *T. gondii* tissue cysts in meat and has demonstrated its potential as an effective intervention [92,93,94].

Studies have reported that *T. gondii* is rendered unviable by irradiation at doses of 0.4–1 kGy [79,82,93,95]. UV irradiation has been shown to inactivate *T. gondii* tachyzoites [96], but recently published protocols are based on prolonged exposure (i.e. up to 60 min) to UV irradiation [97,98]. Overall, the differences between gamma and UV electromagnetic radiations include the origin of production. Gamma rays are produced from the nucleus of energetic atoms whereas UV rays are produced from atomic orbitals with lower energy levels; thus, gamma rays can cause ionization in food media and penetrate deeper than UV irradiation, making it more effective for inactivation of *T. gondii* under similar conditions. Research on the effects of extremely low frequency electromagnetic fields (ELF-EMF) on *T. gondii* by Ozlem-Caliskan et al. showed that pulsed and continuous EMF exposure (75 Hz) reduced the number of *T. gondii* tachyzoites in comparison with the controls [99]. Table 3 shows the positive findings of some studies about the use of IR on *T. gondii*.

6.3. *Curing*

Curing treatments are used to preserve meat by the addition of a combination of salt, nitrates, nitrite or sucrose and low temperature smoking [100,101]. Studies indicate that tissue cysts of *T. gondii* are killed during salt curing, although the inactivation of theses cysts depends on the maturation time, temperature of storage and salt concentration in the curing process [10,101–103]. Hill et al. reported that injection within 8 h of 2.0% NaCl or 1.4% or higher of lactate-based salt solution into pork loins containing infective tissue cysts prevented transmission of *T. gondii* [103]. Lunden and Uggla also showed that the curing of lamb meat with salt and sugar for 64 h at 4°C or smoking salt-injected meat at temperatures not exceeding 50°C for 24–28 h was effective for killing *T. gondii* [100]. Similar results have been reported by researchers as presented in Table 3. Nonetheless, additional studies are required to evaluate the safety of meat products cured under different curing conditions with regard to time and salt and nitrite concentration.

7. Chemical and biochemical methods

The control of *T. gondii* in human foodstuff is important principally to protect the human food chain from contamination by *T. gondii* derived from infected animals. Several complementary strategies have been used to control feed and food contamination which include a range of chemical and biochemical treatments. The principal agents used are organic acids and their salts, solvents and other oxidant compounds, formaldehyde, disinfectants, alcohols and other chemicals, enzymes, food additives and plant essential oils (EO) [120–122]. Many products use blends of agents from the same or different chemical groups to achieve synergistic or combined effects. The present review describes the various modes of action and efficacies of different chemical and biochemical agents delivered in food against *T. gondii* occurring in food or the environment.

7.1. *Disinfection*

At present, two chemicals (chlorine and ozone) are most commonly used to treat food, especially drinking water, because their ability to inactivate *T. gondii* oocysts [123]. Both chlorine and ozone are strong oxidizing agents that can cause cell death through inhibition of enzymatic activity, damage to cells by modifying cellular components, alterations in cell permeability or damage to DNA and RNA [124]. A major advantage for the use of these chemical agents is that they are easier to handle than gaseous chlorine or calcium hypochlorite and require shorter contact times and dosages than chlorine for sodium hypochlorite and ozone, respectively. On the other hand, disinfectants are hazardous waste because they contain halogenated compounds, making it essential to use caution in their use in food to prevent cross-contamination. Principally, disinfectants or sanitizers can only function adequately for preventing cross-contamination of raw fruits and vegetables when the required disinfectant residual is controlled during washing by automated monitoring and dosing of the disinfectant [125].

Wainwright et al. studied the chemical inactivation of *T. gondii* oocysts in water by chlorine and ozone. They exposed *T. gondii* oocysts to 100 mg/L of chlorine for 30 min or 2, 4, 8, 16 and 24 h or 6 mg/L of ozone for 1, 2, 4, 8 and 12 min. The results indicated that neither sodium hypochlorite nor ozone effectively inactivated *T. gondii* oocysts, even when used at high concentrations [126]. Dubey et al. exposed cat feces containing *T. gondii* oocysts to 5.25% aqueous sodium hypochlorite for 24 h, but it did not kill the oocysts [66]. Another study showed that oocysts were not inactivated by ozone after exposure to 9.4 mg min l^{-1} in water at 20°C [110]. The results of some studies have also indicated that oocyst exposure to chlorine is generally limited to 15–30 min [126].

Finch et al. showed that the oocysts of a related chlorine-resistant parasitic protozoan were inactivated (≥99%) following exposure to 3 or 4 mg × 1 min/L of

ozone [127]. Frenkel et al. found that a strong concentration of ammonia (28%) killed all oocysts of *T. gondii* within 10 min and a strong tincture of iodine did so within 30 min [78]. Another study found that 5% and 10% concentrations of ammonia for 30 and 10 min, respectively, inactivated oocysts [66]. Another study found that the use of iodine (7% I2 + 5% KI) for 30 min or 1% to 10% formaldehyde for 24 h eliminated all oocysts [128]. Ito et al. showed that sensitization with 1% Neo Kurehasol (coccidiocidal disinfectant) for 120 min, with 1% Lomasept (coccidiocidal disinfectant) for 10 or 15 min or with 5% Lomasept for 5 min completely inhibited sporulation of unsporulated oocysts. In addition, sensitization with Neo Kurehasol or peracetic acid for 48 h, with ethanol for 24 h or with methanol for 12 h killed the oocysts [121]. A summary of the effect of different disinfectants on *T. gondii* oocysts is listed in Table 4 and is based on the findings of Dubey [31].

7.2. Essential oils

Plant extracts and their essential oils (EO) are widely used as alternative treatments against foodborne parasites, especially *T. gondii*. These extracts may have the potential to decrease the side effects of the toxoplasmosis treatment drugs such as the combination of sulfadiazine and pyrimethamine [130]. Dahbi et al. reported the total absence of intra-cerebral cysts in mice who received thyme EO (20 µg), signifying that they blocked the appearance of the cysts. No abnormalities were observed in the control mice who received the EO of thyme [131]. Other studies on mice have shown that extracts of *Nigella sativa* oil in combination with pyrimethamine had a synergistic effect in the treatment of toxoplasmosis. They reported an increased survival rate and decreases in the parasite density and pathological insult to both the liver and spleen, but *Nigella sativa* oil alone had no direct anti-toxoplasmosis effect [132]. Leesombun et al. reported that *Thai piperaceae* plant extract had the potential to act as a treatment for toxoplasmosis by inhibiting parasitic growth in human foreskin fibroblast cells [130]. Table 5 lists the efficacy of some plant species and their EO against *T. gondii*.

7.3. Other biochemical methods

Studies have evaluated the effect of other biochemical treatments against T *gondii*. Pfefferkorn et al. showed that when intracellular *T. gondii* were treated with a concentration of emimycin (originally isolated from the culture filtrate of a *Streptomyces* species), it partially inhibited parasite RNA synthesis and much less emimycin was incorporated into the RNA than would be predicted by the amount of intracellular emimycin riboside triphosphate [140]. Sonda et al. showed that although aureobasidin A (a cyclic depsipeptide antibiotic isolated from the filamentous fungus *Aureobasidium pullulans* R106) treatment did not induce tachyzoite to bradyzoite stage of conversion in *T. gondii*, it did result in a loss of intracellular structure and vacuolization within the parasite. Moreover, aureobasidin A inhibited sphingolipid synthesis in *T. gondii* [141]. Palencia et al. found that mice infected with *T. gondii* and treated orally with benzoxaborole AN3661 (a boron-containing compound) did not show an apparent increase in disease, while untreated control groups had a significant number of lethal infections.

Table 4. Effect of some disinfectants on *T. gondii* Oocysts.

Disinfections	Concentration	Treatment time (min/h/day)	Effective	Ref.
Formalin	10%	48 hr	No	[121]
Sulfuric acid +dichromate	63/7%	30 min	No	[66]
Ethanol +acetic acid	95/5%	1 hr	No	[66]
	95/5%	24 hr	Yes	
Ammonium hydroxide	5.0%	10 min	No	[66]
	5.0%	30 min	Yes	
Sodium hyporchlorite (Purex)	6.0%	24 hr	No	[66]
Sodium lauryl sulfate	0.1%	24 hr	No	[66]
Cetyl trimethyl ammonium	0.1%	24 hr	No	[66]
Tween 80	0.1%	24 hr	No	[66]
Ammonia, liquid	5.5%	1 hr	No	[78]
	5.5%	3 hr	Yes	
Tincture of iodine	2.0%	10 min	No	[78]
	2.0%	3 hr	Yes	
	7.0%	10 min	Yes	
Aldesol (contains benzalchoniumchloride, glutaraldehyde, and gloxal)	33%	24 hr	No	[129]
Tincture of hibisept (contains chlorhexidine gluconate in ethanol)	-	24 hr	No	[129]
Izosan-G (contains sodium dichloroizicyanurate-dihydrate in granulate)	.02%	24 hr	No	[129]
Lomasept	1%	1 hr	No	[121]
		3 hr	Yes	
Neo Kurehasol	5%	24 hr	No	[121]
Paracetic acid	5%	48 hr	Yes	[121]
Sodium chloride +potassium or sodium lactate	2% and ≥1.4% respectivily	14 days at 4°C	yes	[103]
Chlorination of water	100 mg/L	24 hr	No	[126]
Ozone treatment of water	6 mg/L	12 min	No	[126]
Ozone treatment of water	9.4 mg/L	20 min	No	[110]

Table 5. In vitro and in vivo studies on anti-toxoplasmosis effects of herbal medicine.

Plants and essential oils	Concentration	Result	Ref.
Satureja khuzestanica essential oil	0.2 and 0.3ml/kg	Mortality rate of infected mice was 8 days after oral administration of EO at 0.2 and 0.3 ml/kg.	[133]
Bunium persicum (Boiss) Essential Oil	0.05 and 0.1 mL/kg	Potential of Boiss EO for production of new preventive agent against toxoplasmosis.	[134]
Zingiber officinale (Ginger) extract	500 µg/ml	GE/F1 (fraction 1 obtained from GE) induced anti-*T. gondii* effects inactivating apoptotic proteins in infected host cells through the direct inhibition of *T. gondii* and has antiparasitic properties which inhibited inflammatory cytokine secretion in vivo.	[135]
Myristica Fragrans Houtt. Essential Oil	24.45 µg/mL	In vitro anti-*T. gondii* assay, oil extract caused significant inhibition with EC50 of 24.45 µg/mL.	[136]
Thymus broussonetii Boiss essential oil	20 µg/animal orally	Total absence of intracerebral cysts in mice who received EO of thyme, which appear to block appearance of cysts. No abnormality observed in control mice who received the EO of thyme.	[131]
Psidium guajava L. essential oil	3.94 ± 0.39 µg/mL	In vitro anti-*T. gondii* assay showed that guava leaf EO showed promising EC of 3.94 ± 0.39 µg/mL, as compared to the standard drug clindamycin (EC50 = 6.24 ± 0.53 µg/mL).	[137]
Curcuma longa water extracts	100 and 200 mg/kg/day	Most effective extract was *Curcuma longa* ethanol extract which showed a 98.6 and 99.2% inhibition of growth of *T. gondii* tachyzoites in 100 and 200 doses, respectively, compared to control.	[138]
Curcumin from the plant *Curcuma longa*	12.9 ± 0.5 µM and 38.3 ± 0.9 µM	Curcumin at the tested doses inhibited the enzymatic activity of recombinant TgGlo1 amplified from *T. gondii* cDNA and parasitic propagation of in vitro cultured *T. gondii*. Ki and IC50 were 12.9 ± 0.5 µM and 38.3 ± 0.9 µM, respectively.	[139]
N. sativa oil (NSO) +Pyrimethamine (PYR)	PYR (12.5mg/kg) and NSO (5 ml/kg) body weight/day	NSO+PYR combination markedly improved the antioxidant capacity of *T. gondii* infected mice compared to infected untreated controls. In total, combination of NSO and PYR had synergistic effect in treatment of toxoplasmosis.	[132]

TgCPSF3 (*Toxoplasma* CPSF3: TGGT1-285200) is a promising new purpose of *T. gondii* that provides the opportunity for the extension of anti-parasitic medicine [142]. A previous study reported that benzoxaborole AN6426 inhibits the growth in human cells of *T. gondii* and provided evidence that the target AN6426 in this organism is the LeuRS editing site [143]. Borges *et al*. proved that the BnSP-7 PLA2 (BnSP-7 toxin, a Lys49 phospholipase A2 (PLA2) homologue of *Bothrops pauloensis* snake venom) exerts an anti-toxoplasmosis effect at a lower dose than that needed to induce cytotoxicity in HeLa cells and also modulates the immune response of host cells [144].

Research on reduction of *T. gondii* development through inhibition of parasitic antioxidant enzymes by dinuclear iron (III) have shown that, in the presence of this compound, the redox environment becomes an oxidant in the LLC-MK2 cells. A reduction in catalase and superoxide dismutase activity in the treated parasites and the presence of reactive oxygen species in the parasitophorous vacuoles was observed, indicating an impaired protozoan response against these radicals. These results suggest that this compound disorders the redox balance of *T. gondii*, inducing cystogenesis and parasitic death [145].

Suzuki et al. reported that CD8(+) T cells can remove *T. gondii* cysts by their perforin-mediated cytotoxic activity. They provided a novel mechanism of the immune system against chronic infection with *T. gondii* [146]. Ochiai et al. found that CD8(+) T cells are capable of removing *T. gondii* cysts by recognizing epitopes commonly expressed in type II and III strains of *T. gondii* or cross-reactions between these two genotypes [147].

A review article by Assolini et al. on nano-medicine advances in toxoplasmosis shows that nano-materials smaller than 1000 nm particle in size are currently being investigated as an alternative for the treatment of *T. gondii* infection [148]. Similarly, Adeyemi et al. reported that inorganic gold, silver and platinum nanoparticles (NPs: 0.01–1,000 µg/mL) caused 90% inhibition of *T. gondii* growth with EC50 values of ≤7, ≤1 and ≤100 µg/mL for gold, silver, and platinum NPs, respectively [149]. In an evaluation of the effect of enzymes on *T. gondii*, de Carvalho et al. demonstrated that incubation of macrophages with trypsin significantly inhibited the uptake of *T. gondii* and confirmed that treatment of macrophages with cytochalasin D under such conditions blocks the typical phagocytic process and partially inhibits *T. gondii* infection in the cells [150].

8. Conclusion

The results of research clearly indicates that food contaminated with all structural forms of *T. gondii* (tachyzoites, bradyzoites, oocysts, sporocysts, sporozoites and enteroepithelials) pose a risk to public health if consumed in raw or undercooked meat, unpasteurized milk, raw vegetables and water contaminated with *T. gondii* oocysts from cat feces. Food preservation technologies are based on the prevention if the growth, the inactivation or killing of the microorganism. This review describes all appropriate and applicable methods and their parameters for

effective inactivation, prevention or killing of *T. gondii* in different types of food and tissues. There are other ways to inactivate *T. gondii* that have not been the focus of research thus far. We therefore suggest that further research should evaluate the treatment of food samples with novel thermal or non-thermal technologies and chemical and biochemical processing methods for the inactivation of *T. gondii*. These include PEF, PLT, CP, pulsed UV, tumbling and injection, enzymes, active packaging materials and Hurdle technology. Many technological, economic and regulatory barriers must to be overcome before the food supply can benefit from these methods.

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Disclosure statement

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