Universitat Autònoma de Barcelona

MICROBIAL TRANSGLUTAMINASE: A REVIEW ON CURRENT CONCERNING ASPECTS

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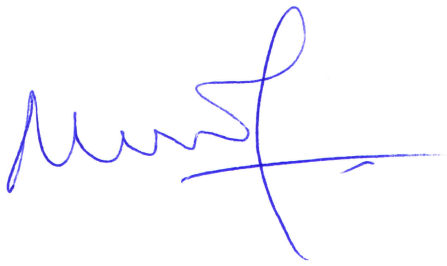
INFORME DEL DIRECTOR DEL TRABAJO

Montserrat Mor-Mur Francesch

Informa que el trabajo titulado "**Microbial transglutaminase: a review on current concerning aspects**" ha sido realizado bajo mi supervisión o tutela por Ioana Darloman, dentro del módulo Trabajo Fin de Máster del Máster Oficial de Calidad de Alimentos de Origen Animal de la Universitat Autònoma de Barcelona.



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1. ABSTRACT

Microbial transglutaminase is an enzyme of the class of transferases, used as a processing aid in food systems. While it does have the advantages of being cost-effective and increasing significantly several technological and functional properties of food products, in the past years there has been an arising debate regarding aspects such as consumer deception and the possible negative health effects of this enzyme in the human body, partially due to the lax legislation and lack of detection methods. Given the interest in this topic, the aims of this review have been to analyze microbial transglutaminase in the current legal context, focusing on health aspects. It is concluded that, at this point in time, more research on the effect of microbial transglutaminase on human health is needed in order to fully confirm or rebut most hypotheses and speculation discussed in this review. One thing is sure, however: analytical methods for the detection of microbial transglutaminase in food products are urgently needed, as well as specific legislation regarding the use, quantities and labelling of food products in which the enzyme has been used.

2. INTRODUCTION

Food additive use in the food industry has been constantly increasing in the last few decades, however, a clear tendency towards *clean labels* has been observed in the last years (Matthias et al., 2016). One food additive is transglutaminase, commonly used in meat products and other foods of animal origin.

Microbial transglutaminase (mTGase) is an enzyme of the class of transferases, known to modify functional properties of protein in food systems. It has the advantages of being cost-effective and increasing significantly the texture, water holding capacity and other technological properties of various food products (Ando et al., 1989) and, since it's considered a processing aid in most countries, it does not need to be mentioned on the label (Kaufmann et al., 2012).

Generally, most studies associated with the application of transglutaminases are focused on the effects of the enzymes on functional and sensory properties, but few types of research are related to the health aspects of this enzyme.

The significant increase in the use of transglutaminases in the last decade, contradictory literature about the health effects of using mTGase in food industry, as well as the existing debate regarding legal regulation, has created an increasing interest in the health advantages and disadvantages of transglutaminase addition to food products.

The current debate on the subject of transglutaminases (TGase) has not only awaken my personal interest to review this topic, but also the Gordon Research Seminar organizes the 5th conference titled "Transglutaminases in Human Disease Processes", which has been held from June 16 to June 17 2018 at Les Diablerets Conference Center, where 48 confirmed speakers will discuss recent discoveries and technological advances.

The aims of this review are the following:

- To compare mammalian and microbial transglutaminase
- To evaluate the use of mTGase as processing aids in the context of current legislation, as well as the importance of detection methods
- To research and describe the health effects of transglutaminase enzymes
- To discuss the many inconsistencies found in the literature in regard to the health effects of both microbial and mammalian mTGase

The methodology followed to elaborate the present analysis was a systematic review of scientific literature on transglutaminases from reliable databases such as PubMed, GoogleScholar, ScienceDirect, Trobador+, Scopus, from TGase origin and production, characteristics and health impact on the human body to current legislation. Mendeley Desktop v1.19.2 software was used to manage all references.

3. CHARACTERISTICS OF TRANSGLUTAMINASES

Transglutaminases (TGases) are enzymes in the class of transferases, highly distributed in nature as they have been found in animal tissues and body fluids (Folk et al., 1980), plants (Falcone et al., 1993) and microorganisms (Ando et al., 1989) (**Table 1**). They were first introduced by Clarke et al. (1959) as enzymes responsible for the transamidating activity of guinea pig liver. Nowadays, TGases are classified under 2.3.2.13 in the ENZYME nomenclature database as protein-glutamine ɣ-glutamyltransferases.

ORGANISM	SPECIES	LOCALIZATION
Mammals	Found in all species	Ubiquitous (keratinocytes, platelets, placenta, epidermis, hair follicles, prostate, lungs, brain, bone marrow, spleen...)
Fishes	*Cirrhiana microlepis* *Pagrus major* *Oreochromis niloticus*	- - -
Amphibians	*Ranidae* and *Bufonidae* families	Epidermis and eggs
Reptiles	*Lacertilia* group	-
Birds	*Gallus gallus domesticus*	Gizzard, epidermis, erythrocytes
Invertebrates	*Limulus* *Brugia malayi* *Caenorhabditis elegans* *Crassostrea gigas* *Penaeus monodon* *Marsupenaeus japonicus* *Pacifastacus leniusculus*	Hemocytes - - Striated adductor muscle - - -
Plants	*Heliantus tuberosus* *Glycine max* *Malus domestica* *Nicotiana tabacum* *Arabidopsis thaliana* *Zeamais*	Chloroplasts Leaves Pollen Flowers - Chloroplasts
Fungi	*Candida albicans* *Phytophthora sp* *Saccharomyces cerevisiae*	-
Microorganisms	*Streptoverticillium sp* *Leishmania sp* *Bacillus subtilis* *Bacillus circulans*	-

Table 1. Organisms expressing enzymes of the transglutaminase family (Adapted from Mariniello et al., 2008)

In 1966, Folk and Cole started researching the isolation and application of enzymes from mammalian tissues and body fluid and, as a result, guinea pig liver TGase was the first and only TGase commercially available until the late 1980s, used as a texture enhancer in foods. However, the high costs of enzyme purification and Ca^{2+} dependency of guinea pig TGase resulted in a loss of interest in potential industrial applications (Yokoyama et al., 2004).

In 1989, Ando et al. isolated TGase from *Streptoverticillium* S-8112, which excreted the enzyme into the cultural broth, making its purification much easier and cost-effective (Seguro et al., 1996). In addition, such TGase is Ca^{2+} independent and shows a lower substrate specificity compared to guinea pig TGase (Yokoyama et al., 2004). The advantages of the newly discovered enzyme made it widely spread as a functional enzyme, used up to this day in many food products.

3.1. Chemical structure of transglutaminases

3.1.1. Microbial transglutaminase

Microbial transglutaminase is a monomeric enzyme with 331 aminoacids in a single polypeptide chain and a molecular weight of approximatively 40kDa.The secondary structure consists of eight β-strands surrounded by 11 α-helices and a single cysteine residue is located at the deep cleft at the edge of the disk-like formation (Ando et al., 1989; Jaros et al., 2006). The cysteine^{64} residue is essential for the catalytic activity of mTGase. The crystal structure of mTGase is showed in **Figure 1**. As described by Kanaji et al. in 1993, a significant loss of activity is observed in the presence of inhibitors such as N-ethylmaleimide, cystamine, monoiodoacetate and a variety of heavy metals.

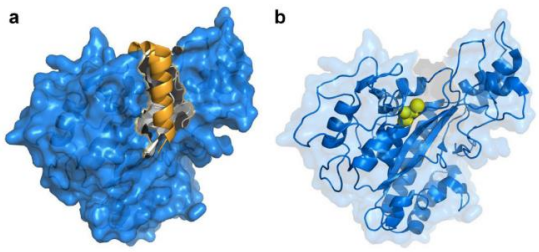

Figure 1. Crystal structure of mTGase. The active site is covered by an a-helix (**a** - gold), which is cleaved upon activation, exposing the active site cysteine residue (**b** – yellow spheres). (Rachel and Pelletier, 2013)

Its isoelectric point is 8.9 and the optimum pH ranges from 6.0 to 7.0 with some residual activity at pH 4.0 and 9.0 The optimal temperature varies depending on pH conditions; at pH=6.0, the optimum temperature is 50ºC. Microbial transglutaminase can retain some

activity even near the freezing point, however, it loses all activity at 70ºC and over (Ando et al., 1989; Seguro et al., 1996; Motoki and Kumazawa, 2000; Yokoyama et al., 2004).

3.1.2. Mammalian transglutaminase

Nine TGase genes have been described from *Homo sapiens* and 8 of them code catalytically active enzymes (**Table 2**). Some common features shared by each member of the mammalian TGase family are the lack of glycosylation and disulfide bonds despite the presence of potential N-linked glycosylation sites and cysteine residue. All TGases lack N-terminal hydrophobic sequence and all members of the TGase family require calcium for the catalytic activity. While the primary structure of TGase enzymes seems to be different, they all share the same amino acid sequence at the active site (Metha and Eckert, 2005). The crystal structure of human TGase2 is showed in **Figure 2**.

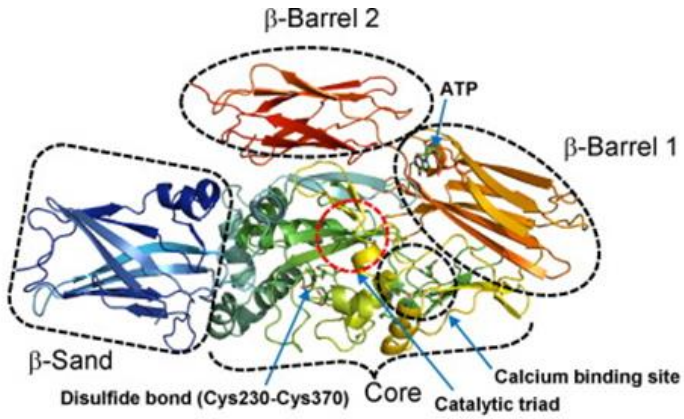

Figure 2. Crystal structure of mammalian TGase2. TGase is shown in ribbon drawing with the β-sandwich domain, the catalytic core domain, and the first and second β-barrel domain. (Han et al., 2010)

TGase 1, TGase 3 and factor XIIIa, are expressed and stored in zymogenic or inactivated forms and can be activated only in the presence of high calcium levels, which are not common and ubiquitous in living cells and their activity is strongly inhibited by the intracellular concentrations of GTP/GDP/GMP and also by ATP (Nemes et. al, 2005).

ENZYME	ALTERNATE NAME	LOCALIZATION	FUNCTION
FXIIIa	Fibrin-stabilizing factor, fibrinoligase, plasma TG	Platelets, placenta, synovial fluid, chondrocytes, astrocytes, macrophages	Blood clotting, wound healing, bone growth
TGase1	TG_1, keratinocyte TG, particulate TG	Membrane-bound in keratinocyte	Cell envelope formation during keratinocyte differentiation
TGase2	Tissue TG, liver TG, endothelial TG, erythrocyteTG, TG_C	Widely distributed in many tissues, cytosolic, nuclear, membrane, extracellular	Apoptosis, cell adhesion, matrix stabilization, cell-survival signaling
TGase3	Callus TG, hair follicle TG, bovine snout TG, TG_E	Hair follicle, epidermis, brain	Cell envelope formation during keratinocyte differentiation
TGase4	Prostate TG, TGp, androgen regulated major secretory protein, vesiculase, DP_1	Prostate	Reproduction, especially in rodents as a result of semen coagulation
TGase5	TG_X	Foreskin keratinocytes, epithelial barrier lining and skeletal muscle	Cornified cell envelope formation during keratinocytes differentiation
TGase6	TG_Y	Testis and lungs	Unknown
TGase7	TG_Z	Ubiquitous, but mainly in testis and lungs	Unknown
B4.2	Band 4.2, ATP binding erythrocyte membrane protein	Erythrocyte membranes, bone marrow, spleen	Major component in erythrocyte skeletal network

Table 2. Mammalian transglutaminases and their characteristics
(Adapted from Mariniello et al., 2008; Metha and Eckert, 2005)

3.2. Reactions catalyzed by transglutaminases

Transglutaminase is able to introduce covalent cross-links by catalyzing acyl transfer reactions between the ɣ-carboxyamide group of peptide glutamine and primary amines, including the Ɛ-amino group of lysine groups, resulting in the polymerization of proteins. If primary amines are not available, water can act as acyl acceptor resulting in the deamidation of the glutamine residue and forming glutamic acid and ammonia (Seguro et al., 1996).

The transglutaminase name is somewhat of a misnomer because these enzymes do not react with the free amino acid of glutamine (Gln); they target the ɣ-carbonylamide function in the side chain of Gln residues in protein substrates. The selection of the particular Gln depends more on its location in the ternary structure of the protein and less on the primary sequence surrounding it. Also, TGases seem to react best with Gln (acceptor) residues in flexible regions of proteins, often in the N and C terminal domains, and always in endo-positions (Metha and Eckert, 2005).

As described by Facciano in 2009, protein substrates for TGases can be divided in two main families:

- protein substrates acting as acyl donor; those who contain the reactive glutamine
- protein substrates acting as acyl acceptor; those who contain the reactive lysine

Sometimes, a protein TGase substrate may contain both reactive glutamine and lysine residue. The availability and the number of these reactive residues represent the biochemical features leading to dimer or polymer formation by cross-linking reaction catalyzed by TGase (Facciano, 2009).

The acyl transfer reaction can be used to introduce amino acids or peptides into a protein, such as improving the methionine and lysine content of casein or soybean proteins, as described by Ikura et al. in 1981 and Nonak et al. in 1996. In addition, microbial transglutaminase can be used to incorporate new amino acids to proteins and peptides, which will behave like native proteins (Motoki and Kumazawa, 2000), making TGase a powerful tool for enhancing the nutritive value of foods and also the modification of functional properties, as for example, solubility, emulsifying capacity, gelation properties and other (Jaros et al., 2006).

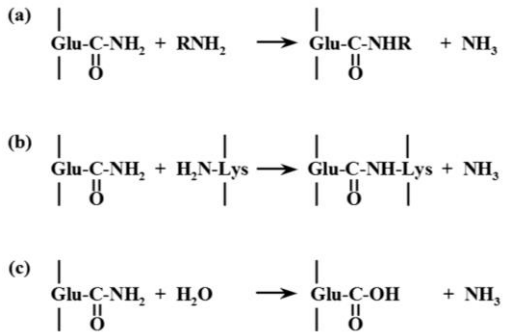

Figure 3. Reactions catalyzed by transglutaminase. **a** - acyl transfer, **b** - crosslinking of Gln and Lys residues in proteins or peptides resulting in an ε-(γ-glutamyl)lysine (G- L) bond, c – deamidation (Yokoyama et al., 2004)

4. TRANSGLUTAMINASES IN THE FOOD INDUSTRY

Microbial transglutaminase can modify functional properties of food proteins by amine incorporation, cross-linking, and deamidation and adhering to the bonding surfaces of foods such as meat, fish, eggs, and vegetables as a thin layer exhibiting strong adhesion in small amounts (Santhi et al., 2015). It acts as a beneficial protein-binding agent due to its functional properties that improve the texture and gelation of mechanically treated meat products, dairy products, plant-based patties and sausages, etc. (Ahmed et al., 2007) Some of the main uses of mTGase in food products are summarized on **Table 3**.

PRODUCT	FUNCTION
Meat (restructured meat, hamburger, meatballs, sausages…)	Improved rheological properties, water holding capacity, appearance, hardness
Milk (creams, drinks, desserts, dressing…)	Higher stability and better texture
Fish (paste, restructured products…)	Improved rheological properties, water holding capacity, appearance, hardness
Yogurt	Higher gel formation and stability, lower syneresis
Bakery products	Higher volume and improved texture
Plant protein products	Gel formation with similar texture to animal protein
Soy (tofu, mapo doufu)	Improved shelf-life and texture
Gelatin-based sweets	Low calorie desserts with improved texture and elasticity

Table 3. Main applications of microbial transglutaminase in food products
(Adapted from Amirdavani et al., 2018)

4.1. Meat products

Microbial transglutaminase can produce restructured meat by binding together small pieces of meat. Kuraishi et al. first developed in 1996 a meat binding system using mTGase and caseinate simultaneously. When caseinate reacts with mTGase, it becomes viscous and functions as a glue to bind different protein-based foods together. Using this system, large pieces of restructured lean such as beefsteaks or fish fillets can be produced from fragments (Yokoyama et al., 2004).

Over the years, many studies have proven the technological effects of mTGase on meat products. As for example, in 1998, Hammer reported that addition of 0.2% of mTGase in finely minced sausages increased hardness and firmness, suggesting occurrence of meat protein linking during the mixing of raw batter results in a finer protein network structure once the product is cooked. Microbial transglutaminase effectively enhanced the texture of chicken breast patties and reduced the cooking loss (Uran et al., 2013). In beef gels, mTGase improved the water holding capacity (Pietrasik and Li-Chan, 2003). In porcine myofibrillar protein, mTGase improved the emulsification activity index and decreased the creaming index, which resulted in improved long-term emulsion stability, especially at pH values above 6.0, although significant increases were found at all pH (Hong and Xiong, 2012). Bak et al. (2012) prepared minced cured restructured ham using mTGase, combined with high-pressure (600 MPa) treatment without affecting the physicochemical characteristics of the ham, especially color. In 2010, Romero de Avila et al. recommended the use of mTGase in liquid or powder form to manufacture restructured dry-cured ham from deboned pork leg.

One of the main goals of many food companies has been the production of foods with clean labels, by eliminating or reducing additives such as salt and/or phosphates. Studies have shown that a reduction in said substances would alter juiciness, texture and shelf life in meat products (Trespalacios and Pla et al., 2007a). The use of mTGase in salt/phosphate reduced meat products has been studied by many researchers. In 1995, Nielsen et al., demonstrated that mTGase would indeed counter the effects of such reduction without affecting the texture. In phosphate-free low salt restructured pork shoulder, cooked at 72ºC for 65min, with the previous addition of 0,15 % mTGase, consistency and juiciness was significantly improved compared to a control without mTGase (Dimitrakopoulou et al., 2005). In low-salt dry-cured hams, Fulladosa et al. (2009) substituted NaCl with potassium lactate and 2g of mTGase/kg raw muscle, obtaining a good binding without affecting color, flavor or texture.

4.2. Fish products

In 1990, Seki et al. found that endogenous fish TGase caused hardening fish protein paste at low temperature by crosslinking. Both endogenous fish TGase and exogenous mTGase could improve the efficacy of fish raw materials by increasing crosslinking. There is still controversy over whether the endogenous fish TGase is the only factor in fish hardening. It seems that mTGase treatment maintains and improves the texture of fish products, however,

the quality of the final product is highly dependent on the freshness of the raw materials (Yokoyama et al., 2004).

4.3. Dairy products

Milk casein, which does not gel even when heated, is a very good substrate for mTGases, which convert it into a heat-resistant, firm gel. (Sharma et al., 2002) Yogurt has the disadvantage of serum separation. The addition of mTGase can overcome this problem by improving the water holding capacity of the gel. Microbial transglutaminase is also used to produce dairy products with low fat content or reduced content of non-fat solids. (Jaros et al., 2006).

4.4. Other

Soy proteins, such as 11S and 7S globulins, are also adequate substrates for the mTGase reaction. Tofu is prepared by the coagulation of soybean proteins with the addition of Ca^{2+} and Mg^{2+} and/or glucono-δ-lactone. It is very difficult to produce long-life tofu since its texture can easily be altered by sterilization.The addition of mTGase improves the texture during long-time storage of sterilized tofu. (Yokoyama et al., 2004).

Treatment of noodles and pasta with mTGase prevented the deterioration of texture after cooking and improves the strength of the products even when low-grade flours are used. Also, loaf volume of bread was maintained or improved in the presence of mTGase when certain ingredients were substituted or reduced (Sakamoto et al.,1996).

5. LEGISLATION

5.1. Legislation in the EU

Microbial transglutaminase is considered by the European Parliament Directive 2000/13 EC a processing aid and not an ingredient, and therefore does not need to be listed in the ingredients of the finished product. There is no specific legislation about minimum or maximum quantities allowed to be added to food products, but, as other processing aids, it is recommended to add the minimum quantity needed to achieve that function in the processing of food. According to the Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, those meat or fish products which have been reconstituted, must include the word "formed" or "restructured" on the label. This description informs the consumer that a product which appears to be a whole piece of meat or fish, actually consists of different pieces combined together by other ingredients. It is not, however, specific to the use of transglutaminase since it includes food additives, food enzymes and other means.

5.2. Legislation in the USA

Microbial transglutaminase has been recognized as safe (GRAS) by the Food Drug Administration (FDA) in 1998 for use to improve texture and cooking yields in various meat and poultry products and as protein cross-linking agent to fabricate or reform cuts of meat. Regarding labeling, USDA's Food Safety and Inspection is responsible for regulating the labeling of mentioned food products; those which have been formed from pieces of whole muscle meat, or that have been reformed from a single cut, must be include such information as part of the product name with the word "formed". The enzyme must also be listed in the ingredient list, along with any other ingredients used in the product. Contrary to European legislation, transglutaminase is not considered a processing aid that would be exempt from labeling.

5.3. Legislation in Australia and New Zealand

Microbial transglutaminase is regulated by the Australia New Zealand Food Standards Code (FSC) and is included in Clauses 3 to 18 of Standard 1.3.3. Processing aids. Processing Aids are substances used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but do not perform a technological function in the final food. Also, processing aids must be used at the lowest level necessary to

achieve a function in the processing of that food. Although most processing aids have a maximum permitted level, it is not the case mTGase or other enzymes of microbial origin, included in the clause 17 of the Standard 1.3.3. Regarding labeling, Processing Aids are not required to be included in the ingredients list.

5.4. Legislation in other countries

In May 2014, there was released the "Labeling foodstuffs made with the enzyme transglutaminase" report by Ajinomoto (one of the main producers and distributors of mTGase), which indicated that microbial transglutaminase is a processing aid and under current law, shall not be labeled in the list of ingredients.

6. HEALTH EFFECTS OF TRANSGLUTAMINASE

6.1. The role of native transglutaminase in the human body

6.1.1. Cell death, cell surviving signaling and cancer

Among the various types of transglutaminase described so far, TGase2 which is also referred to as the cytosolic, type II, or liver transglutaminase, is a unique member of the transglutaminase enzyme family. Calcium-dependent activation of TGase2 has been implicated in diverse biologic functions, such as differentiation, receptor-mediated endocytosis, cell adhesion, and induction of apoptosis. However, more recent studies have provided direct evidence that increased expression of TGase2 can prolong cell survival by preventing apoptosis It has been proposed that proapoptotic and antiapoptotic effects of TGase2 vary widely depending on its location within the cell. In view of these findings regarding cell growth, cell survival and metastasis, many researchers have speculated that TGase2 expression in cancer cells promotes signaling events that could affect not only the adhesive, migratory, and invasive functions of tumor cells but also their growth and survival. (Metha et al., 2005).

6.1.2. Neurodegenerative disorders

Many studies have reported that TGase activity is involved in the pathogenesis of Alzheimer's disease. More than 20 years ago, Selkoe et al. proved that TGase activity contributed to the formation of protein aggregates in Alzheimer's Disease (AD). In brains of patients with AD, protein cross-links occur, leading to increased products of reactions catalyzed by TGase. In many people with Huntington disease (HD), an increase in transglutaminase-catalyzed lysine bonds has been observed (Metha and Eckert, 2005).

6.1.3. Celiac disease

In celiac disease, characterized by debilitating intestinal and systemic manifestations, TGase2 is the main target of autoantibodies, and symptoms in the related skin disease: dermatitis herpetiformis are caused by immune complex deposits of TGase3 (Metha and Eckert, 2005).

Multiple mTGase linked proteins, including those in bakery products, are immunogenic to celiac disease patients. Many studies have shown that gluten-sensitive individuals are currently on the rise (Gerrard and Sutton, 2005). Lerner and Matthias (2015) studied the use

of mTGase in celiac disease foods was investigated. They concluded that mTGase cross-linking of gluten may be hazardous in celiac patients, however, no study has concluded that the use mTGase in products without gluten can result in gluten-like proteins which may trigger an immunologic response.

6.2. Possible health effects of exogenous transglutaminase in food products

Most negative health effects described in scientific literature have been, under my review, the result of confusion between human and microbial TGase. Many authors have hypothesized a possible health effect of residual mTGase ingested through food, which, once in the human body, can mimic endogenous TGase. If so, and considering that mTGase is not Ca^{2+} dependent, which is a limiting factor in the catalytic activity of mammalian TGase, which would be the effects of this exogenous enzyme on the human body? Should we be concerned?

To my best knowledge, in most cases the enzyme is denaturalized and thus, loses its activity due to the thermal conditions to which most products are treated before commercialization. In those cases where some mTGase may remain in the final product, said denaturalization would occur due to the low pH of gastric acid.

I believe, however, based on the research that I have reviewed, that the main focus should be not on the enzyme itself, but on its products and by-products. All three possible reactions catalyzed by transglutaminases have NH_3 as a by-product, which, in high enough quantities could have negative effects on human health. Moreover, as a result of mTGase action, it is possible to obtain aminoacid sequences which may trigger an immunologic response in sensitive individuals.

6.3. Bioavailability of cross-linked proteins

Microbial transglutaminase forms both inter- and intra-molecular covalent bonds of glutamine and lysine. Many questions have been raised regarding nutritional aspects linked with digestibility of such cross-linked peptides and the bioavailability of lysine that was incorporated.

After ingestion of cross-linked proteins, the dipeptide (G-L) is cleaved by the activity of γ-glutamylamine cyclotransferase, a kidney enzyme, and γ-glutamyl transpeptidase, located in the intestinal brush-border membrane, in the kidneys and blood. (Jaros et al., 2006). Seguro et al. (1995) reported that the second enzyme bisects the G-L isopeptide directly to lysine and glutamate. Since lysine is an essential amino acid, it is believed it would be nutritionally

beneficial. Seguro et al., (1996) concluded after in-vivo experiments that rats fed with casein treated with mTGase had no abnormalities compared to a control group.

Motoki and Seguro (1998) found that the only difference between mTGase-modified proteins and native proteins is the number of links between lysine and glutamine residues.

6.4. Health advantages of microbial transglutaminase

The growing demand for healthier products with nutritional properties has been constantly increasing in the later years. There are various strategies to be used in order to achieve these nutritional foods, such as changes in the use of raw materials, reformulation of products or using enzymes. Even though microbial transglutaminase has been used traditionally in the food industry because it significantly improves sensory properties, there are many evidences that prove its potential for also increasing nutritional and functional properties (Kieliszek and Misiewicz, 2013).

One interesting area for using mTGase is the development of new products or reformulation of traditional meat products such as hamburgers or sausages with protein from plant sources, reducing costs by substituting part of myofibrillar proteins with soy or pea protein, while maintaining textural properties. Various studies have been conducted to evaluate the efficiency of mTGase in improving interactions and gel forming capacity of meat proteins with non-meat proteins. In 2003, Ramirez-Suarez and Xiong determined that mTGase could cross-link soy and muscle proteins producing a firm gel. In a 3:1 mixture of myofibrillar/pea protein, mTGase greatly improved gel strength, indicating that G-L cross-linking occurred between muscle and pea protein (Luciano and Arntfield, 2012).

Martinez et al. (2011) formulated beef patties enriched with polyunsaturated n-3 fatty acids and dietary fiber with optimal texture and minimal effect on color and cooking loss by a pre-treatment with mTGase. Muguruma et al. (2003) produced chicken sausages with soybean protein, casein, whey protein isolate. They showed that cross-linking soy protein isolate, casein, whey protein isolate and myofibrillar proteins with mTGase improved heat stability and emulsifying properties, resulting in a better texture compared to the control without mTGase.

Cross-linking of chicken myofibrillar proteins with globular proteins of egg catalyzed by mTGase, in combination with high pressure treatment at 500 MPa for 30 min at 40ºC improved the binding properties, texture and color (Trespalacios and Pla, 2007b).

7. DETECTION METHODS

Even though the use of mTGase as a protein cross-linking agent to reform cuts of meat are considered as generally safe, some safety aspects of the restructuring of meat using mTGase and other applications of the enzyme in food products are currently under discussion. Firstly, consumer deception is taking place when a restructured meat is not properly labeled. Also, during the restructuring process it is possible that microbial contaminations from the surface of the smaller meat pieces to the interior of the final piece may occur. It is necessary to have analytical methods for the detection of mTGase in food products. In the last years, histological techniques have been used to detect mTGase treated meat products (Kaufmann et al., 2012). However, these methods only allow the detection of structural changes in the meat, but do not provide specific information concerning the use or nonuse of binding agents, nor the type of agent (e.g. fibrinogen-thrombin, alginate, or mTGase).

Kaufmann et al., 2012 speculated that the protein extract of mTGase used in meat products may contain *Streptoverticillium mobaraensis* DNA which may have not been completely lost during the industrial production of mTGase. In order to detect possible DNA residues, real-time PCR was used. The main limitations of this technique are the following: DNA is only an indication of *S. mobaraensis*, but not of the mTGase itself. Moreover, during commercial mTGase production, the content of the DNA is reduced by dilution, as well as during the production of the final products and so, PCR is not expected to have the required sensitivity to screen the final meat products for mTGase. It is considered a suitable technique to confirm that, once mTGase is detected, it is not a ubiquitous TGase, but it is indeed coming from *S. mobaraensis*.

In 2017, Jira et al. developed a highly specific HPLC–MS/MS-method for the detection of mTGase with and without caseinate in restructured pork, beef, chicken, and turkey by using tryptic marker peptides. The detectability of mTGase in restructured meat pre-treated under various conditions (raw, heated, oil marinade, emulsion marinade, seasoning salt, and breadcrumbs) was compared and no significant differences between the treatments were observed.

The method developed by Jira et al., 2017a and 2017b allows the detection of transglutaminase with lower detection limits compared to the one by Kaufmann et al., 2012. It

also allows simultaneous detection of casein by using two marker peptides, which is a useful tool regarding the allergenicity of these milk proteins. However, both methods are only able to obtain qualitative results, but cannot determine the amount of residual mTGase or degree of cross-linking.

8. CONCLUSIONS

Besides the aspect of consumer deception, possible health impairments for celiac disease patients and a potential risk of microbial contaminations, microbial transglutaminase is currently also discussed as an allergen.

Several hypotheses have been proposed in regard to the health effects residual transglutaminase or products and by-products of the enzyme, yet, at this point in time, more research on the mTGase effect on human health is needed in order to fully confirm or rebut most hypotheses discussed in this review. The arising interest has also created a large amount of speculation in the scientific community, leading to confusion in some cases. There has been published contradictory information, most of which is the result of attributing functions and characteristics of mammalian transglutaminase to microbial transglutaminase.

As reported by Kaufmann, whom attended IFFA 2016 (Germany), an international platform for the meat processing industry, several exhibitors offered mTGase. My own experience at FoodTech 2018 (Spain) has been similar; various companies were offering mTGase, showing the great demand for this enzyme in the meat industry and indicating that meat binding is a common practice. Therefore, analytical methods for the detection of microbial transglutaminase in meat and meat products are needed, as well as specific legislation regarding the use, quantities and labelling of food products in which mTGase has been used.

9. REFERENCES

Ahhmed AM, Kawahara S, Ohta K, Nakade K, Soeda T, Muguruma M. 2007. Differentiation in improvements of gel strength in chicken and beef sausages induced by transglutaminase. Meat Sci. 76(3):455–462. doi:10.1016/J.MEATSCI.2007.01.002.

Amirdivani S, Khorshidian N, Fidelis M, Granato D, Koushki MR, Mohammadi M, Khoshtinat K, Mortazavian AM. 2018. Effects of transglutaminase on health properties of food products. Curr Opin Food Sci. 22:74–80. doi:10.1016/J.COFS.2018.01.008.

Ando H, Adachi M, Umeda K, Matsuura A, Nonaka M, Uchio R, Tanaka H, Motoki M. 1989. Purification and Characteristics of a Novel Transglutaminase Derived from Microorganisms. Agric Biol Chem. 53(10):2613–2617. doi:10.1080/00021369.1989.10869735.

Clarke DD, Mycek MJ, Neidle A, Waelsch H. 1959. The incorporation of amines into protein. Arch Biochem Biophys. 79:338–354. doi:10.1016/0003-9861(59)90413-8.

Dimitrakopoulou MA, Ambrosiadis JA, Zetou FK, Bloukas JG. 2005. Effect of salt and transglutaminase (TG) level and processing conditions on quality characteristics of phosphate-free, cooked, restructured pork shoulder. Meat Sci. 70(4):743–749. doi:10.1016/j.meatsci.2005.03.011.

Facchiano F, Facchiano A. 2005. Transglutaminases and Their Substrates. In: Transglutaminases. Vol. 38. Basel: KARGER. p. 37–57.

Falcone P, Serafini-Fracassini D, Del Duca S. 1993. Comparative Studies of Transglutaminase Activity and Substrates in Different Organs of Helianthus tuberosus. J Plant Physiol. 142(3):265–273. doi:10.1016/S0176-1617(11)80421-9.

Folk JE, Cole PW. 1966. Mechanism of action of guinea pig liver transglutaminase. I. Purification and properties of the enzyme: identification of a functional cysteine essential for activity. J Biol Chem. 241(23):5518–25.

Folk JE, Park MH, Chung SI, Schrode J, Lester EP, Cooper HL. 1980. Polyamines as physiological substrates for transglutaminases. J Biol Chem. 255(8):3695–700.

Fulladosa E, Serra X, Gou P, Arnau J. 2009. Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. Meat Sci. 82(2):213–218. doi:10.1016/j.meatsci.2009.01.013.

Gerrard JA, Sutton KH. 2005. Addition of transglutaminase to cereal products may generate the epitope responsible for coeliac disease. Trends Food Sci Technol. 16(11):510–512. doi:10.1016/J.TIFS.2005.07.002.

Hammer, G. F. 1998. Microbial transglutaminase and diphosphate in finely comminuted cooked sausage. Fleischwirtschaft, 78(11), 1155–1156, 1159–1162, 1186.

Han B-G, Cho J-W, Cho YD, Jeong K-C, Kim S-Y, Lee B Il. 2010. Crystal structure of human transglutaminase 2 in complex with adenosine triphosphate. Int J Biol Macromol. 47(2):190–195. doi:10.1016/J.IJBIOMAC.2010.04.023.

Hong G-P, Xiong YL. 2012. Microbial transglutaminase-induced structural and rheological changes of cationic and anionic myofibrillar proteins. Meat Sci. 91(1):36–42. doi:10.1016/J.MEATSCI.2011.12.002.

Ikura K, Yoshikawa M, Sasaki R, Chiba H. 1981. Incorporation of Amino Acids into Food Proteins by Transglutaminase. Agric Biol Chem. 45(11):2587–2592. doi:10.1080/00021369.1981.10864924.

Jaros D, Partschefeld C, Henle T, Rohm H. 2006. Transglutaminase in dairy products: chemistry, physics, applications. J Texture Stud. 37(2):113–155. doi:10.1111/j.1745-4603.2006.00042.x.

Jira W, Schwägele F. 2017a. A sensitive high performance liquid chromatography–tandem mass spectrometry method for the detection of microbial transglutaminase in different types of restructured meat. Food Chem. 221:1970–1978. doi:10.1016/J.FOODCHEM.2016.11.148.

Jira W, Schwägele F. 2017b. A sensitive HPLC-MS/MS method for the simultaneous detection of microbial transglutaminase, and bovine and porcine fibrinogen/thrombin in restructured meat. Food Chem. 237:841–848. doi:10.1016/J.FOODCHEM.2017.06.030.

Kanaji T, Ozaki H, Takao T, Kawajiri H, Ide H, Motoki M, Shimonishi Y. 1993. Primary structure of microbial transglutaminase from Streptoverticillium sp. strain s-8112. J Biol Chem. 268(16):11565–72.

Kaufmann A, Kö R, Widmer M. 2012. Determination of microbial transglutaminase in meat and meat products. Food Addit Contam Part A. 29(9):1364–1373. doi:10.1080/19440049.2012.691557.

Kieliszek M, Misiewicz A. 2014. Microbial transglutaminase and its application in the food industry. A review. Folia Microbiol (Praha). 59(3):241–250. doi:10.1007/s12223-013-0287-x.

Kuraishi C, Yamazaki K, Susa Y. 2001. Transglutaminase: its utilization in the food industry. Food Rev Int. 17(2):221–246. doi:10.1081/FRI-100001258.

Luciano FB, Arntfield SD. Use of transglutaminases in foods and potential utilization of plants as a transglutaminase source-Review. Rev Biotemas. 25(4):1–11. doi:10.5007/2175-7925.2012v25n4p1.

Mariniello, L, Di Pierro, P, Giosafatto, CVL, Sorrentino ARP. 2008. Transglutaminase in food biotechnology. Res Signpost. 37/661(2):185–211.

Martinez, B., Miranda, J. M., Franco, C. M., Cepeda, A. and Vazquez, M. 2011. Evaluation of transglutaminase and caseinate for a novel for- mulation of beef patties enriched in healthier lipid and dietary fiber. LWT-Food

Matthias T, Jeremias P, Neidhöfer S, Lerner A. 2016. The industrial food additive, microbial transglutaminase, mimics tissue transglutaminase and is immunogenic in celiac disease patients. Autoimmun Rev. 15(12):1111–1119. doi:10.1016/j.autrev.2016.09.011.

Mehta K, Eckert R. 2005. Transglutaminases: Family of Enzymes with Diverse Functions. Progress in experimental tumor research. 38. doi: 10.1159/000084229

Motoki M, Seguro K. 1998. Transglutaminase and its use for food processing. Trends Food Sci Technol. 9(5):204–210. doi:10.1016/S0924-2244(98)00038-7.
Motoki M, Kumazawa Y. 2000. Recent Research Trends in Transglutaminase Technology for Food Processing. Food Sci Technol Res. 6(3):151–160. doi:10.3136/fstr.6.151.

Muguruma M, Tsuruoka K, Katayama K, Erwanto Y, Kawahara S, Yamauchi K, Sathe SK, Soeda T. 2003. Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. Meat Sci. 63(2):191–7.

Mycek MJ, Clarke DD, Neidle A, Waelsch H. 1959. Amine incorporation into insulin as catalyzed by transglutaminase. Arch Biochem Biophys. 84(2):528–540. doi:10.1016/0003-9861(59)90613-7.

Nemes Z, Petrovski G, Csosz E, Fesus L. 2005. Structure-Function Relationships of Transglutaminases ? A Contemporary View. In: Transglutaminases. Vol. 38. KARGER. p. 19–36.

Nielsen PM. 1995. Reactions and potential industrial applications of transglutaminase. Review of literature and patents. Food Biotechnol. 9(3):119–156. doi:10.1080/08905439509549889.

Nonaka M, Sawa A, Matsuura Y, Motoki M, Nio N. 1996. Deamidation of Several Food Proteins Using Free and Immobilized Ca2+ -Independent Microbial Transglutaminase. Biosci Biotechnol Biochem. 60(3):532–533. doi:10.1271/bbb.60.532.

Pietrasik, Z., Li-Chan, E. C. Y. 2002. Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. Food Research International, 35(4), 387–396

Rachel NM, Pelletier JN. 2013. Biotechnological applications of transglutaminases. Biomolecules. 3(4):870–88. doi:10.3390/biom3040870.

Ramirez-Suarez JC, Xiong YL. 2003. Rheological properties of mixed muscle/nonmuscle protein emulsions treated with transglutaminase at two ionic strengths. Int J Food Sci Technol. 38(7):777–785. doi:10.1046/j.1365-2621.2003.00731.x.

Romero de Ávila MD, Ordóñez JA, de la Hoz L, Herrero AM, Cambero MI. 2010. Microbial transglutaminase for cold-set binding of unsalted/salted pork models and restructured dry ham. Meat Sci. 84(4):747–754. doi:10.1016/J.MEATSCI.2009.11.011.

Sakamoto H, Yamazaki K, Kaga C, Yamamoto Y, Ito R, Kurosawa Y. 1996. Strength enhancement by addition of microbial transglutaminase during Chinese noodle processing. Nippon Shokuhin Kagaku Kaishi 43:598–602

Santhi D, Kalaikannan A, Malairaj P, Arun Prabhu S. 2017. Application of microbial transglutaminase in meat foods: A review. Crit Rev Food Sci Nutr. 57(10):2071–2076. doi:10.1080/10408398.2014.945990.

Seguro K, Kumazawa Y, Kuraishi C, Sakamoto H, Motoki M. 1996. The Ɛ-(ɣ-glutamyl)lysine moiety in crosslinked casein is an available source of lysine for rats. J Nutr. 126(10):2557–2562. doi:10.1093/jn/126.10.2557.

Seki N, Uno H, Lee NH, Kimura I, Toyoda K, Fujita T, Arai K. 1990. Transglutaminase activity in Alaska pollack muscle and surimi, and its reaction with myosin b. Nippon Suisan Gakkaishi 56:125–132

Selkoe DJ, Abraham C, Ihara Y. 1982. Brain transglutaminase: in vitro crosslinking of human neurofilament proteins into insoluble polymers. Proc Natl Acad Sci U S A. 79(19):6070–4.

Sharma R, Zakora M, Qvist KB. 2002. Susceptibility of an industrial α-lactalbumin concentrate to cross-linking by microbial transglutaminase. Int. Dairy J. 12: 1005–1012.

Shleikin AG, Danilov NP, Ternovskoy G V. 2011. Modification of food products properties by use of transglutaminase. Procedia Food Sci. 1:1568–1572. doi:10.1016/J.PROFOO.2011.09.232.

Trespalacios P, Pla R. 2007a. Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. Food Chem. 100(1):264–272. doi:10.1016/J.FOODCHEM.2005.09.058.

Trespalacios P, Pla R. 2007b. Synergistic action of transglutaminase and high pressure on chicken meat and egg gels in absence of phosphates. Food Chem. 104(4):1718–1727. doi:10.1016/J.FOODCHEM.2007.01.077.

Uran, H, Aksu, F, Yilmaz, I, Durak, MZ. 2013. Effect of transglutaminase on the quality properties of chicken breast patties. Kafkas Univ. Vet. Fak. Derg. 19:331–335.

Yokoyama K, Nio N, Kikuchi Y. 2004. Properties and applications of microbial transglutaminase. Appl Microbiol Biotechnol. 64(4):447–454. doi:10.1007/s00253-003-1539-5.