TRANSGLUTAMINASE IN FOOD ADULTERATION AND PERSPECTIVES OF SUSTAINABLE DEVELOPMENT

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Abstract. *Transglutaminase (TGase) is an enzyme which creates isopeptide bonds between side chains of amino acids glutamine and lysine of various proteins, thus forming crosslinks and joining protein molecules together. It is widely used in food industry. In particular, it improves the texture and appearance of e.g. meat products, increases the product weight and extends the shelf life. TGase is also used for replacement of preservatives, emulsifiers, stabilizers, flavors, dyes and sweeteners. Usage of TGases from various sources is regulated by governing bodies and is currently allowed in European countries and in Ukraine. However, utilization of TGases in manufacturing chain is generally not disclosed on the product labels. For instance, food companies prefer to not disclose e.g. that the ham block has been manufactured out of small pieces of meat leftovers using TGase. They charge buyers as if that was the solid section of real meat. Moreover, ordinary consumers generally have little ideas on the processing technology of the food products they buy. Most of the product label information is written in a complicated way using abbreviations. By avoiding decoding and proper explanations, manufacturers try to mislead consumers and avoid responsibility. Furthermore, manufacturers constantly apply tricks to replace food ingredients and use various additives. This may lead to aggravating global problem of food adulteration and falsification. The goal of this paper was to draw attention of various circles of health professionals, regulatory officials and wide public to possible involvement of TGases in our food products.*

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Introduction. The famous phrase “We are what we eat” is thought to be first coined by the ancient Greek philosopher and physician Hippocrates. He believed that food plays an important role in human life because it was the source of energy and nutrients (Drees and Barthel, 2022). It is hard to disagree with such statement. However, the current situation on the food market may be not that clear. These days food contains less useful and natural ingredients and more artificial additives due to the constant profit apetites of manufacturers. This is referred to as food product adulteration. Transglutaminase, also known as “meat glue”, has become one of the most valuable instrument for food manufacturers. The natural role of TGases is formation of biological polymers, which are necessary for the body to create barriers and stable structures. Due to the ability of the TGases to form bond between proteins, it can literally glue together meat, seafood, fish and dairy products (Calcaterra et al., 2020). The usage of TGase for industrial applications was fist initiated in Japanese food industry back in 1959 by the company «Ajinomoto» under the trade name Activa. Buckwheat noodles and crab meat were the first products created via TGase. These days the usage of TGases became a lucrative business for the food companies, as products glued together from different pieces or from food industry wastes look just like naturally prepared and sold at a higher price. Currently, this enzyme is widely used and we can often see food products manufactured via its usage on the shelfs of our supermarkets. However, we generally do not realize the involvement of TGases in the processing chain of the product. Moreover, most manufacturers do not indicate the presence of TGase in the detailed chemical composition of their products since this information is not obligatory due to current official requirements.

For instance, widely used and popular in Ukraine "crab sticks" are in fact produced out of leftovers of fish products and have nothing to do with crabs. Moreover, transglutaminase is used in so called "molecular cuisine" restaurants with non-traditional dishes. Combination of soy isolate with transglutaminase improves the passage of probiotics and prebiotics through the stomach to the lower intestines (Yew et al., 2011). It is widely believed, at least by food companies, that transglutaminase is completely safe for the consumers. However, given the catalytic properties of the enzyme and its ability to form isopeptide bonds between proteins, it is possible that TGases in food products can affect health and well being of the consumers even in marginal quantities. Consequently, we become increasingly dependent on consumption of low quality food ingredients as they become a substantial part of our diet under false believe that they were the high quality products.

We set up a project to estimate how widespread the TGase usage is in the Ukrainian food industry and whether manufacturers always inform consumers about the presence of TGases in their products.

TGase is used not only in food processing chain. It is utilized e.g. in drug development and to increase synergistic activity and bioavailability of probiotics. The TGases used in food industry are mostly obtained from plants and microorganisms of the family *Streptomyces spp* (Vasić et al., 2023). The bacterial enzyme is calcium-independent and it is widely used in food industry. Another example of TGase is coagulation factor XIII (fibrin-stabilizing factor). It is activated by thrombin and makes crosslinks between polymer fibers of fibrin, leading to stabilization of blood clot. This TGase is normally extracted from blood of cows, chickens and pigs.

The main advantages which food producers get by using the TGase enzyme from the point of view of the producers of this supplement (https://cpt.in.ua/ua/catalog/transglyutaminaza/) are the following:

- improved consistency of food products;
- increased juiciness and elasticity of products;
- raise in the output of final products;
- ensure stable quality;
- reduction in moisture loss especially during storage;
- minimal quantities of enzyme required for processing chain due to high enzymatic activity of the additive.

The most used in food industry variant of transglutaminase these days is microbial transglutaminase (mTGase) obtained from the bacteria *Streptoverticillium moboarense*. It is considered safer than the TGases from other sources. It has higher binding properties than TGases from blood plasma and other alternative sources. This feature has positive effect on the functional biocatalytic properties of the enzyme, and enables to efficiently modify proteins in food products at the molecular level. As a result, usage of mTGase has a positive impact on important physicochemical properties of food proteins (hydrophobicity, gelation, etc.). Therefore, it is used in food industry to reduces a large number of other ingredients in recipes, such as carrageenans, gums, phosphates, emulsifiers, etc. (Yew et al., 2011).

Application of transglutaminase in various branches of food industry in Ukraine

Meat industry. TGase is used to process meat derived ingredients from various sources, including muscles, cartilages, blood serum, intestines, tissues from various organs, etc. These mixtures can be combined with vegetable proteins, milk, muscular (myoglobin) proteins, or collagen protein. As it was stated above, transglutaminase is a biocatalyst that ensures formation of the protein bonds due to cross-links between amino acids, namely lysine and glutamine, and therefore can form solid blocks of meat products which resemble the natural meat. It is widely used in production of e.g. ham and sausages. The techniques which utilize TGases can be so advanced that the meat industry is able to produce a solid piece of meat which is difficult to distinguish from the real meat. Technically, the TGases contribute to formation of a protein mesh structure between the cells of which moisture and fat are retained. For this, a certain concentration of the protein is required. It is estimated that for efficient protein-protein crosslinking the total concentration of proteins in the processed row materials should be at least 12% in the mixture. The nature of the protein generally does not have substantial influence on the ability of TGase to form crosslinks. Often TGase is used for gluing small pieces of meat into one bigger block to give it a shape of a natural solid piece of meat, as shown in Fig. 1. In this figure a ham bought from a supermarket in Ukraine was sliced and left in the refrigerator overnight. During this time, the pieces of meat developed areas of different colors. We assumed that this ham block was produced by gluing smaller pieces of low quality meat by using transglutaminase. The label of that ham package did not contain any indication of using TGases for in the processing chain of the product. Fig. 2 shows typical product obtained via crosslinking activity of TGases, namely “crab sticks”. The label on the product did not contain any indication on the usage of TGases in processing chain and neither it clearly indicated source of the ingredients used in manufacturing the product. However, the simple comparison of the size of the “crab sticks” with real crabs which we could have seen in nature does not support the idea that these “crab sticks” could theoretically be produced out of solid crab pieces. Moreover, the price of the product does not support the idea that the final product was produced using crab derived ingredients.

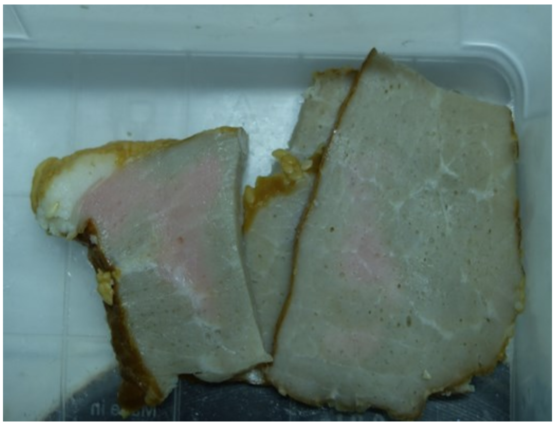

Fig. 1. This ham block was most probably manufactured from small pieces of meat from various source glued together by transglutaminase.

Fig. 2. This product, so called "crab sticks", was most probably produced from fish wastes via transglutaminase.

Dairy industry. Milk proteins have been the subject of extensive research and are considered one of the most extensively studied protein complexes found in food. They exhibit a diverse range of functionalities and play important roles in various aspects of food science and technology. Individual milk proteins, such as caseins and whey proteins, have unique properties that contribute to their functional capabilities in food formulations. Transglutaminase is often used in manufacturing of dairy products to improve texture, increase stability and enhance functionality. It can help create a firmer and smoother texture in dairy products like cheese, yogurt and ice cream. It can also be used to bind different components together, such as in the production of processed cheese or restructured dairy products. It was shown that modification of milk proteins by transglutaminase leads to their partial cross-linking and formation of high molecular weight polymers in the range of 55–200 kDa. Enzymatic modification of milk proteins increases the ethanol stability of both raw milk and pasteurized milk but not thermal stability of the milk (Tarapatskyy M, 2019). Overall, the use of transglutaminase in dairy products offers several advantages. Here are some of the benefits:

1) Improved texture: transglutaminase can enhance the texture and mouthfeel of dairy products by cross-linking proteins. It helps to form a network of proteins, resulting in a smoother and more cohesive texture. This is especially useful in products like cheese, yogurt, and ice cream.

2) Increased stability: transglutaminase improves the stability of dairy products by creating stronger bonds between proteins. This helps to maintain product integrity and prevent separation or syneresis. It can improve the heat stability of dairy proteins, making them more resistant to thermal processing.

3) Extended shelf life: the use of transglutaminase can enhance the shelf life of dairy products. It can improve the firmness and reduce the moisture content of products, which inhibits the growth of spoilage microorganisms and extends product freshness.

4) Enhanced functional properties: transglutaminase can improve the functional properties of dairy proteins. It can increase the water-holding capacity, emulsifying properties, and gelation abilities of proteins. This is particularly beneficial in processed dairy products like cheese or yogurts, where these properties are important for product quality and stability.

5) Reduced fat and sodium content: transglutaminase enables the production of dairy products with reduced fat and sodium content. By utilizing transglutaminase, it is possible to create low-fat or reduced-fat dairy products without compromising texture and sensory attributes.

It is however important to note that the use of transglutaminase in dairy products should adhere to regulatory guidelines and labeling requirements to ensure consumer safety and transparency. Moreover, the use of transglutaminase in dairy products should be clearly indicated on the label, especially for individuals with gluten sensitivity or celiac disease, as some forms of transglutaminase are derived from wheat.

Storage of vegetables and fruits. Whey protein modified by TGase in combination with pectin and other ingredients was used in research to create protein film, which could be used for vegetables and fruits to improve their freshness and extend their shelf life (Marquez et al., 2017). One of the most important functions of edible coating is to protect fruits from moisture loss and improve their capability for storage. Moreover, this can help to decrease losses associated with transportation, distribution, and long-term storage on trade shelves. Edible films have been used since 1980. Only 10 vegetable and fruit companies provided such services. Nowadays the use of edible films spreads quickly with ever increasing annual sales exceeding 100,000,000 $ (Tiamiyu et al., 2023).

Baking industry. In baking, transglutaminase can be used to enhance the elasticity and strength of dough. It can improve the dough's handling properties, allowing it to be stretched and shaped more easily. TGase is also useful to retain moisture without loss of mass fraction. The beneficial properties of TGase are extensively exploited in baking industry in the production of bread, pizza dough, and other yeast-based products, as well as in manufacturing of pasta and noodles. The use of TGase brings substantial advantages also to products baked out of frozen dough (Kim et al., 2008). TGase can also be used to create layered or laminated dough. By bonding together layers of dough, it can help create flaky and tender pastries, such as croissants and puff pastry. Another application of transglutaminase in the baking industry is in the production of gluten-free products. Since gluten is responsible for the structure and elasticity in traditional baked goods, gluten-free products often lack these qualities. In gluten-free baking, the absence of gluten can also result in a dense and crumbly texture. Transglutaminase can help to improve the texture and structure of gluten-free dough, especially those which are based on non-gluten species of flour such as rice, buckwheat and corn, making it more similar to traditional baked goods (Diowksz and Sadowska, 2021). TGase is also intensively exploited in development of new varieties of protein rich and allergen free bread such as soy bread (Hutasoit, 2012). Overall, use of TGases can lead to improved texture and a more enjoyable eating experience for individuals on a gluten-free diet. It is worth noting that the use of transglutaminase in the baking industry is subject to various types of regulations and guidelines in various countries. It is important for bakers to follow the recommended usage levels and ensure that they comply with any labeling requirements related to the use of transglutaminase in their products.

Public awareness. Generally, ordinary consumers are mostly unaware that they consume products containing TGases. We set out to investigate how widespread TGases are in the Ukrainian food industry and whether manufacturers always inform consumers about the presence of TGases presence in their products.

It is important to note, that by solely organoleptic studies, which involve standard methods of evaluating the product quality using the senses, it is difficult to establish the presence of TGases in cheese, milk, yogurt, etc. As to the safety of the products manufactured via use of TGase, the most investigators conclude that TGases have no unwanted side effects. However, increasingly more information is emerging about the occurrence of diseases caused by excessive consumption of recently emerged products. Thus, serological markers of microbial and tissue TGases have been detected. They can bind gliadin with the formation of antibodies to TGase, which is known as being involved in celiac disease (Torsten and Aaron, 2018; Agardh, 2020).

It is the microbial transglutaminase that is still most often used in the food industry as it was in the early days of its usage (De Jong and Koppelman, 2002; Lerner and Benzvi, 2021). It was reported that mTGase can potentially suppress the body's defense mechanisms and mimic other types of human transglutaminase, which might cause chronic human diseases. It was assumed that food additives such as transglutaminase extensively used in the food products is one of the major environmental factors for induction of autoimmune diseases. Thus, TGases can increase intestinal permeability through the opened tight junctions, resulting in entry of foreign immunogenic antigens and activation of the autoimmune cascade. Similar evidences were reported to increase the intestinal permeability TGases (Amirdivani, 2018). It was stated that TGases might represent a new environmental inducer of celiac disease (Aaron and Torsten, 2019). Moreover, TGases can be immunogenic in celiac patients and thus can trigger abnormal immune responses. That leads to the load increase on the intestinal lumen of the immune system (Matthias et al., 2016). The etiology of these diseases is related to epidermal transglutaminase, which acts as a trigger for epithelium damage. Based on the above adverse effects, regulatory and food safety authorities should review the status of transglutaminase. Food must be labeled and carefully evaluated for toxicity and health safety. Currently, there is no clear information on product labels which type of TGase was used for food manufacturing. Moreover, in Ukraine most of information on product labels is written in a complicated and incomprehensible manner for ordinary consumers. This is one way for the manufacturers avoid responsibility. According to regulations in many EU countries, food manufacturers are obliged to provide a detailed information about composition of their products, so that consumers can make the reasonable informed decisions to consume the products or avoid them. Therefore, the EU consumers are generally protected from potential unscrupulous actions of market operators. One form of "meat glue" was banned in Europe, namely the combination of thrombin and fibrinogen, both of which are obtained from the serum of animals (European Parliament, 2010). The binding properties of this additive however is different from mechanism of TGase, as it works via polymerization of fibrin molecules after thrombin activates fibrinogen and transfers it to fibrin. In addition, European Parliament stated that Commission Directive itself recognized that the use of "meat glue" (from thrombin and fibrinogen in this case) as a food additive could mislead the consumer as to the state of the final food, taking into account that the purpose of this additive was to bind individual pieces of meat to obtain a single meat product (European Parliament, 2009). Therefore, the risk of misleading consumers is obvious (Deweid et al., 2019). It is of note that the sole purpose of the use of TGase in meat processing industry does not differ from the use of the banned "meat glue" based on thrombin and fibrinogen.

64 years have passed since the isolation of TGase. During this period, a massive of different information was found about positive and negative consequences of its usage for human health. It is now clear that TGases are able to cross-link proteins and other macromolecules, changing their structure, composition, antigenicity, physical and chemical characteristics. There is also data supporting cautious approach towards wide usage of TGases, as they can manifest themselves as the causes of allergic reactions, poisoning, malabsorption syndrome, problems with the pancreas, etc.

It is still preliminary to draw a firm conclusion about positive or negative effects of transglutaminase as a food additive. Careful and thorough investigation of the role and consequences of the use of TGases in technological food chains is still largely required in European countries and in Ukraine.

Conclusion: Without any doubt, the use of transglutaminase in the food industry brings many advantages. It improves organoleptic indicators, increases effectiveness of food production, promotes creation of the new products and increases profitability of food producers. It is still allowed in Ukraine and other European countries as a replacement of preservatives, emulsifiers, stabilizers, flavors, dyes and sweeteners. On the other hand, there is increasing amount of data supporting involvement of TGases in pathogenesis of many diseases. To this, it is still not clear whether the technological approach to create higher value products out of low quality ingredients is ethically acceptable and feasible in terms of requirements for sustainable development. Moreover, a thorough public discussion is still needed to figure out if this could be regarded as a counterfeit product when the new technology allows to glue together pieces of meat wastes to make it look like a natural piece of meat and sold at a higher price. However, it is clear that ordinary consumers should have chances to be informed about what they buy and consume.

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