

ASSESSMENT OF THE E 920 ADDITIVE (L - CYSTEINE) IN RELATION TO SOME PROBLEMS OF MODERN FOOD INDUSTRY

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Abstract

This paper aims to assess the current state of knowledge about the use of L - cysteine in food industry, regarding certain cultural, legal, technological, toxicological, and other aspects that influence the attitude of the consumers towards food. Use of L - cysteine and its derivatives in bakery allows the optimizing of the technological characteristics of flours and their higher recovery, by using products with high added value. Use the E 920 additive in human food is subject to the cultural and religious controversy, due to the generalized process of obtaining this additive from animal products (keratin). Our study shows that these controversies will be overcome when industrial fermentative technologies of L - cysteine production will be generalized in the market. There exist no data on the potential toxicity of L - cysteine in the usual doses which are used in the baking industry. The only threat to the status of E 920 as a safe additive is the excitotoxic potential, suggested in several recent studies. Also, there exists a potential for extending the use of L - cysteine in the food industry in order to reduce the contamination degree with certain chemicals having carcinogen potential, such as acrylamide and mycotoxins.

Key words: bread, controversy, L - cysteine, safe food

INTRODUCTION

A brochure entitled "25 Amazing Facts about food" was published in 2011, under the signatures of Mike Adams and David Guiterrez and the copyright of Truth Publishing Inc. This brochure makes several statements about some additives used in food industry; that have enjoyed significant notoriety in the Romanian online environment. Thus, the authors make the following statements about one of the additives used in the baking industry: *While some L-cysteine is directly synthesized in laboratories, most of it is extracted from a cheap and abundant natural protein source: human hair. The hair is dissolved in acid and L-cysteine is isolated through a chemical process. Other sources of L-cysteine include chicken feathers, duck feathers, cow horns and petroleum byproducts [1].*

This paper aims to assess the current state of knowledge about the use of this amino acid in nutrition, regarding the most important aspects which influence the market, namely:

cultural, legal, technological, toxicological, etc.

MATERIALS AND METHODS

In order to assess the main aspects of using L - cysteine aminoacid, there have been extensively studied a series of bibliographic databases including the European Commission's legislative, public opinion experts EFSA scientific articles in the fields of food technology, biochemistry, toxicology, nutrition, medical science market research, and opinions of Jewish religious texts scholars.

RESULTS AND DISCUSSIONS

Cysteine has been isolated in 1810 by Wollaston from kidney stones. It is found in variable proportions in different plant and animal proteins (0.8% in zein, 2.3% in wheat gliadin, 12.5% in insulin, 1.1% in muscle proteins, etc.).

L-cysteine and its derivatives are widely used in baking, in quantities up to 90 ppm. Effects of cysteine addition to flour are obvious in the increasing of the fluid properties of dough: it decreases its ability of accumulating energy, toughness and resistance to extension, and increases its adhesiveness and extensibility [2].

Flours which require the addition of reducing agents generally give products with small volume, dense core, undeveloped porosity and poor flavor [16].

There is an intense controversy in the Western world on the use of L-cysteine in bakery. It is not based on the potential adverse effects to human health, but on the assumption that its origin is opposed to the vegan food philosophy and the Muslim Sharia requirements. Mosaic worship expressed through an analysis made by a rabbi, an interesting opinion on the use of L - cysteine in diet.

L-cysteine was considered "kosher" provided that it does not come from human cadavers or have previously been the subject of a religious sacrifice [17].

Some properties of foods rich in cysteine were known and used since talmudic times. Gemara (one of the books of Talmud) relates that Rav Abbah (175-247) using a recipe based on chicken macerate for medicinal purposes and, in the Middle Ages, Rambam (1135-1204) prescribed to Prince Al- Afdal from the court of Saladin a similar recipe for asthma treatment (treatment of this disease is also in use today, using derivatives of cysteine) [17].

Despite this, many Hebrew internet sites consider food containing L - cysteine as "non-kosher".

L-cysteine is not the only food additive in this situation. Similar controversies concern the gelatin, rennet used in the cheese industry, bone powder used to whiten sugar sporadically, frosting agents such as shellac and beeswax, and red colors of **cochineal**.

L-cysteine global market is estimated at 5000 tons per year and is supplied as a result of a mixture of different production technologies, which include keratin hydrolysis, chemical synthesis and fermentation (Fig. 1) [6, 13].

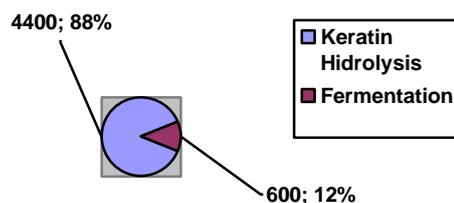


Fig. 1 Global market of L - cysteine (tonnes)

Although for most amino acids there have been developed biosynthetic technologies, the major route for obtaining L cysteine, L - Leucine, L - histidine and L - tyrosine remained the extraction from protein hydrolysates. As for cysteine, it is used the extraction from hydrolyzed keratin, obtained from human hair or feathers of animals [22].

Among the biosynthesis technologies used on an industrial scale we emphasize the biosynthesis technology used by Ajinomoto Co., Inc., Japan, which involves the asymmetric hydrolysis **DL - 2 - amino-Δ2 - thiazolin - 4-carboxylic acid** to L - cysteine, using enzymes secreted by bacteria of the genus *Pseudomonas*. Also, Wacker Chemie (2001) implemented an industrial method for obtaining L - cysteine via a mutant *E. coli*, using glucose as the main carbon source [14]. Market value of L - cysteine is estimated at 55 million dollars, with a growth rate of 4% per year [14]. Approximately 30% of the worldwide produced L - cysteine is used to obtain its pharmacotherapeutic derivatives (N-acetylcysteine and S-carboximetilcysteine, both having mucolytic action).

In addition to the use of L - cysteine as flour treatment agent in bakery industry, is it also used extensively in food animal production industry. Its role in animal feed isto enrich the beef flavor of these products. A lower but constant consumption is recorded in the cosmetic industry, as a basic preparate in anti-aging creams.

Of all the L - cysteine produced worldwide, in 2004, only 12% was obtained by fermentation processes. Annual growth rate for the market share of microbial L-cysteine was estimated at 10%.

The conventional process of cysteine

obtaining involves the acid hydrolysis ($\text{HCl} > 20\%$) of keratin at high temperatures (100°C for 6 hours). The process has certain environmental implications, which are difficult to manage. In order to obtain one kilogram of cysteine, there are required 27 kilograms of hydrochloric acid; this involves the use of big water resources. Comparitively, the biosynthesis method applied by Wacker Chemie requires only 1 kilogram of hydrochloric acid to obtain one kilogram of L - cysteine. Despite this, L - cysteine obtained by fermentation is more expensive than the conventional one and is preferred primarily by pharmaceutical and cosmetic industries. This industries are able to transfer these costs to products with higher added value than food industry.

One of the factors that determine the interest of the pharmaceutical industry for microbial L - cysteine is the fear, deeply rooted in the psychology of consumers, on the risks involved in the consumption of certain products of animal origin, having the capacity of transmitting prion diseases such as Creutzfeldt - Jakob disease (spongiform encephalopathy).

The European Commission experts have analyzed this issue in 2000, at the request of some producers' organizations in the cosmetics industry. They asked for the changing of the Directive 76/768/EEC which prohibited the use of cells, tissues or products of human origin in the manufacture of cosmetics sold in the Common Market, so that the amino acids obtained by acid hydrolysis of keratin can still be used.

The experts' conclusion was that amino acids obtained by hydrolysis of keratin can be considered safe for human consumption, as long as they are not contaminated with other substances with high risk of transmission of human spongiform encephalopathies (TSEs). The conclusion was based on the following assumptions, which can be extrapolated to food: prions associated with TSE have never been identified in hair; the obtaining process involves a series of inactivation conditions that reduce the number of infectious units at a lower level even than the level already agreed by the EU legislation regarding manufacturing

of food gelatine; severe hydrolysis conditions in combination with mandatory tests, regarding the lack of peptides in L - cysteine based preparates, can guarantee the exclusion of transmissible prion protein from them [26]. The European Commission experts were called to respond later (2006) to questions about the safety of adding this amino acid to biscuits for young children (3 to 12 months). Although this amino acid has been approved for use in treating flour, the assessments considering it toxicologically acceptable, it was not mentioned in the list of food additives accepted in the food formulation for babies and toddlers. Biscuits for children should have a controlled content of carbohydrates and fat. But this fact leads to increased fragility of biscuits, inducing additional risk of suffocation. Use of L - cysteine or its hydrochloride derivative product enables optimization of textural characteristics of crunchy products and reduces the mentioned risk.

The usual dose of L - cysteine is max. 1 g / kg flour. Extrapolated to the average quantities of biscuits consumed daily by a toddler, the amount of cysteine intake is the equivalent to 6 mg / day. This amount represents only 2% of the total cysteine provided to a child by his entire daily diet (296 mg).

Consequently, the group of experts concluded that the use of L - cysteine in baby products present no risk to their safety and that is not in contradiction with previous recommendations of the European Commission.

Recommendation stated that the addition of amino acids in the diet of children should be performed only in order to improve its nutritional value [25].

Previous explanation can be applied for exposure adults to this amino acid after the consumption of bakery products. Accurate assessment of the amounts of L - cysteine or its derivatives, provided by the mill - bakery food chain is quite difficult. On one hand, L - cysteine is added to the mill, and on the other hand, improvers used in bakeries contain also varyous amounts of L - cysteine. We suspect a linear correlation between the value of this consumption and the quality of Romanian crops, in which the addition of L-cysteine per

kilogram of flour is unlikely to exceed an average of 0.05 to 0.1 grams. Quantities of L - cysteine may be much higher for biscuits which have dough rheological characteristics adjusted to the required values for the manufacturing processes, by treatments with proteases and reducing agents. The average daily consumption of bread per capita was estimated by Econtext.ro for 2010 to 0.26 kg [19]. If we assume that the average specific consumption is 0.75 kg of flour per kilogram of finished product, then the amount of L - cysteine corresponding to this consumption is 9.75 to 19.5 milligrams. Namely, the amount of L-cysteine is 25 - 50 times lower than the usual daily amount of L-cysteine or its derivatives used for therapeutic purposes, for treating various diseases (fig 2).

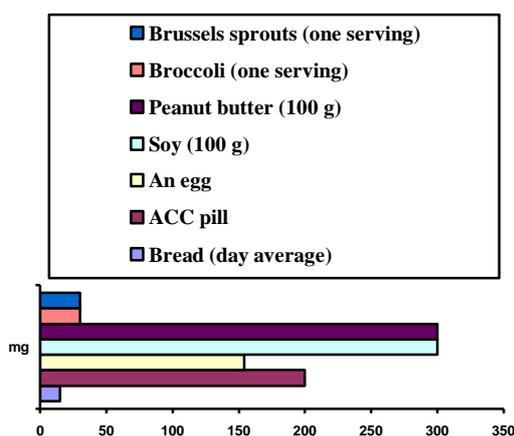


Fig. 2 Daily doses of L - cysteine associated with several components of the diet or medication

New directions for the use of L-cysteine in the food industry.

In recent year there have been patented several methods which use L - cysteine or thiol compounds for reducing the acrylamide content in foods. Acrylamide is a potential carcinogenic substance that is formed when starchy foods are baked, boiled or roasted.

Flukiger and Salih suggested in 2009 a method for treating food to be cooked with an alkaline solution of cysteine or glutathione. Thiol compounds in alkaline solution react with acrylamide, through the carbon double bonds in its molecule, forming a new nontoxic compound [11]. Barry et al. suggested previously a similar method, involving food contact before the heat treatment with a

solution composed from calcium chloride and L - cysteine [3].

Other studies have shown similar effects of inhibition of the acrylamide formation in aqueous systems and *in vivo*, when certain embryos were exposed to high concentrations of acrylamide [8, 18, 7].

Since thiol compounds are potent nucleophiles, they have a strong affinity for the unsaturated electrophilic centers of different dietary toxicants in food, such as aflatoxins, sesquiterpenes, lactones (elephantropin and parthenin), urethane, carbonyl compounds, quinones and even , halogenated compounds [6]. Boyacioğlu et al. published in 1993 a study showing that in particular L - cysteine has a moderate decrease in DON content of whole wheat bread. Along with sodium bisulphite ammonium phosphate, treatment with L - cysteine ensured a reduction of DON content between 38 and 46% [5]. A cysteine derivative, namely N-acetylcysteine at a dose of 800 mg / kg of forage, was able to reduce the toxic effects of aflatoxin B, when added to chickens diet [21].

Cysteine and glutathione are part of the substances able to stop the formation of nitrosamines produced endogenously in the stomach and digestive tract, such as: N-nitrosodimethylamine, N-nitroso-N-nitrosopiperidin and N-nitrosopyrrolidine [20].

Toxic effects of L - cysteine in the body.

Potential toxic effects of L-cysteine have been tested on rats since the '70s. The results published in the *Journal of the Science of Food and Agriculture*, showed that despite feeding rats with bread made from flour treated with doses of 10 to 100 times higher than usual doses, there have not been identified histopathological changes or injury in the body to fifth generation [12].

However, some studies have shown that L - cysteine may have negative effects on the animal organism when it is administrated in very high doses. Dilger et al. introduced feed treated with 30 g / kg L-cysteine in chickens' diet and identified certain changes in their health status, as well as metabolic acidosis and increased oxidative stress in the body [9].

In a previous study, they investigated the effects of supplementing the diet with extreme amounts of sulfur amino acids in several species. They found that excess L - cysteine in doses of 7.5 times higher than dietary recommendations may result after five days of dosing in increased mortality by 50%. In all variants of administration (2.5 - 10 times more than the dietary recommendations), taking L - cysteine suppressed the growth of chickens.

In young mice or pigs receiving similar doses of L - cysteine, were not identified significant changes in mortality, but growth has been drastically suppressed. For none of the three analyzed species there have not been related similar symptoms to cystine excess in food [10].

In the literature there are cited a series of L-cysteine interferences with the therapeutic medication, administered in case of illness. Thus, L - cysteine potentiates the activity of the drugs used to lower blood pressure. It also can cause suppression of immunity, thereby increasing susceptibility to certain diseases [27].

There have been reported situations where L - cysteine inactivated the insulin given to diabetics. However, it is considered that L - cysteine potentiates the toxicity of sodium monoglutamate, for the Chinese restaurants disease sufferers [28]. For the patients suffering from cystinuria, the administration of L- cysteine has a negative effect.

Several views include L - cysteine in excitotoxins category. These are substances that have physiological functions of neurotransmitters (usually amino acids or derivatives thereof), and can supraactivate specific receptors in the nerve cell when they have high concentrations. The process consists of an excessive stimulation of nerve cells, which can result in their destruction. The best known substance with excitotoxic effect is sodium monoglutamate.

In 1990 *Olney et al.* found that systemic administration of L-cysteine to immature mice correlates with destruction of nerve cells in the cerebral cortex, hippocampus, thalamus and striatum. The author's opinion is that neurotoxicity of L - cysteine is also mediated

by glutamate receptors (subtype N-Methyl-D-aspartate) and its magnitude increases in the presence of bicarbonate ions at physiological concentrations [15].

The chemical reactivity of the thiol group of L - cysteine can induce toxicity by autooxidation, through formation of reactive oxygen species [23].

Many doubts about the toxic potential of L - cysteine are induced by associating the high level of of homocysteine in the body with some neurodegenerative and cardiovascular diseases. Homocysteine is involved in many transsulfuration reactions (such as cysteine synthesis in the body), remethylation (in the synthesis of methionine), transmethylation of DNA, proteins and lipids, biosynthesis of certain neurotransmitters or hormones. Homocysteine oxidizes many analogues of L - glutamate (L -homocysteinsulfonic acid, homocysteic acid) and L - aspartate (L-cisteinsulfonic acid, cisteic acid) that exert excitotoxic effects even greater than the excitotoxic effect of homocysteine [4]. Homocysteine enhances LDL autooxidation lipoprotein particles, thus initiating thrombosis or atheroma plaque buildup.

CONCLUSIONS

1. Use of L - cysteine and its derivatives in bakery allows the optimizing of the technological potential of flours and their superior use, by products with high added value. Controversies surrounding the use of additives in food are primarily cultural and religious controversies and are likely to be exceeded when the fermentative technologies for producing L - cysteine will generalize in the market.
2. There is potential to extend the use of L - cysteine in the food industry, to reduce contamination with potentially carcinogenic number of substances such as acrylamide and mycotoxins.
3. There are no data on the potential toxicity of L - cysteine on the usual doses which are used in the baking industry. In medicine, L - cysteine and its derivatives are used in multiple therapies of body detoxification.

4. The excitotoxic potential of L - cysteine is the biggest threat to the "sure additive " status of L - cysteine. Although the mechanisms of glutamate receptors present on nerve cells are not well understood, the number of studies that gather evidence in favor of excitotoxic potential of L - cysteine is growing. However, it is not yet clear to what extent the quantities of L - cysteine added to food during processing, can interfere in these mechanisms.

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