GLUCURONIC ACID FROM FERMENTED BEVERAGES: BIOCHEMICAL FUNCTIONS IN HUMANS AND ITS ROLE IN HEALTH PROTECTION

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ABSTRACT
An increasing rate of morbidity in the world is due to widespread chronic degenerative ailments such as cancer, cardiovascular and neurodegenerative diseases. One of the causes of that is toxification of human organism by xenobiotics and insufficient activity of fat-soluble endobiotics. The present article discusses important theoretical aspects of glucuronidation - the general concept of detoxication and the biochemical mechanism of glucuronic acid conjugation. The way of obtaining fermented beverages with a high content of glucuronic acid by applying the Kombucha symbiotic cultures originated from various parts of the world is demonstrated. The initial hypothesis on the synthesis of glucuronic acid as a characteristic property of natural associations of bacteria and yeasts has been confirmed. It can be prospective for human health protection and chronic diseases prevention. The use of glucuronic acid-containing microbially fermented products in medicine, and their beneficial biological effects on human health are overviewed.

Keywords: *glucuronic acid, uridine diphosphate, detoxication, glucuronidation, Kombucha symbiosis.*

1. INTRODUCTION

Toxification is one of the most extensively debated health issues of the modern age. There are many types of toxins influencing human health such as increased amount of xenobiotics, toxins of infectious microorganisms and reactive metabolites. Insufficient detoxification causes "metabolic poisoning" – an accumulation of toxic metabolites that are not processed by the liver and excreted within cells, tissues and organs. Metabolic poisoning can originate kidney failure and inefficient liver functions, thus leading to the accumulation of toxins in blood and impairment of the functions of brain cells, which originates disturbances of the central nervous system. A permanent contamination with xenobiotics and endogenous toxins, and increasing damage of oxidative stress cause fast progressing of non-communicable diseases (NCD) such as cardiovascular, neurodegenerative, chronic respiratory and kidney diseases, diabetes mellitus type 2 and cancer - the problems affecting affluent societies.

Glucuronic acid (GlcUA**)** is well known as a significant detoxicant in the prophylaxis of human health. UDP-glucuronosyltransferases (UGTs) - the family of enzymes responsible for glucuronidation with many isoforms and wide substrate specificity, allow conjugating GlcUA with various natural and foreign compounds to form glucuronides that are excreted via urine or faeces. Hepatic detoxification is basically aimed at taking fat-soluble materials and making them more polar and water-soluble, so they can be transported and excreted from the body. Such is a common metabolic pathway that usually facilitates excretion. Bacteria and yeasts utilize GlcUA for a number of essential functions. This article overviews GlcUA present in fermented beverages obtained by using Kombucha symbiotic culture from distant parts of the world (France, Tunisia, Serbia, Iran, India, Indonesia, Korea, Japan, Sudan, USA). It permits the possibility of a difference in the composition of symbionts and therefore the active ingredients and their concentration in fermented final products can be different as well. The interest about the production of GlcUA for food application has increased significantly during the last decade. Microbiologists are intensively carrying out target studies on a possibility to enhance the production of GlcUA by changing the Kombucha fermentation medium and process variable parameters.

2. GLcUA: SYNTHESIS AND FUNCTIONS IN THE HUMANS

2.1. Synthesis of GlcUA in the human liver

GlcUA is synthesized from glucose – a carbohydrate that is the primary source of energy for cells; it can be considered as a modified form of glucose in which the alcohol group (-CH_2OH) at the 6^{th} position is replaced with a carboxylic acid (-COOH). Sugars modified in this way are called uronic acids. GlcUA ($C_6H_{10}O_7$) is a derivative of glucose with a molecular mass of 194,14 Da. In human tissue, it is produced by dehydrogenation of uridine diphosphate (UDP) glucose (Figure 1). The first step is the formation of glucose-6-phosphate, its isomerisation to glucose-1-phosphate, and the activation of glucose-1-phosphate to form UDP-glucose, after oxidised into UDP-GlcUA by NAD^+ and UDP-glucose dehydrogenase. Two molecules of NAD^+ are used for each molecule of UDP-GlcUA being formed. GlcUA is highly-soluble in water. In addition to the glucose hydroxyl groups, GlcUA has the

functionality of a carboxylic acid that is ionised at physiological pH and, so, GlcUA can serve as a handle for specific excretion membrane transport systems. UDP-GlcUA is mainly utilised in biosynthetic reactions that involve the condensation of GlcUA with a variety of molecules to form an ether (glucuronide), an ester or an amide. [1]. Normally produced by a healthy liver, GlcUA is the most powerful natural detoxifier (Figure 2, a). Since the liver has an epithelium that actively absorbs numerous substances from the blood, metabolises and secretes them into bile or urine, transporting is an integral part of detoxification. That is a fact that often receives less attention than the biochemical transformations aforementioned [2].

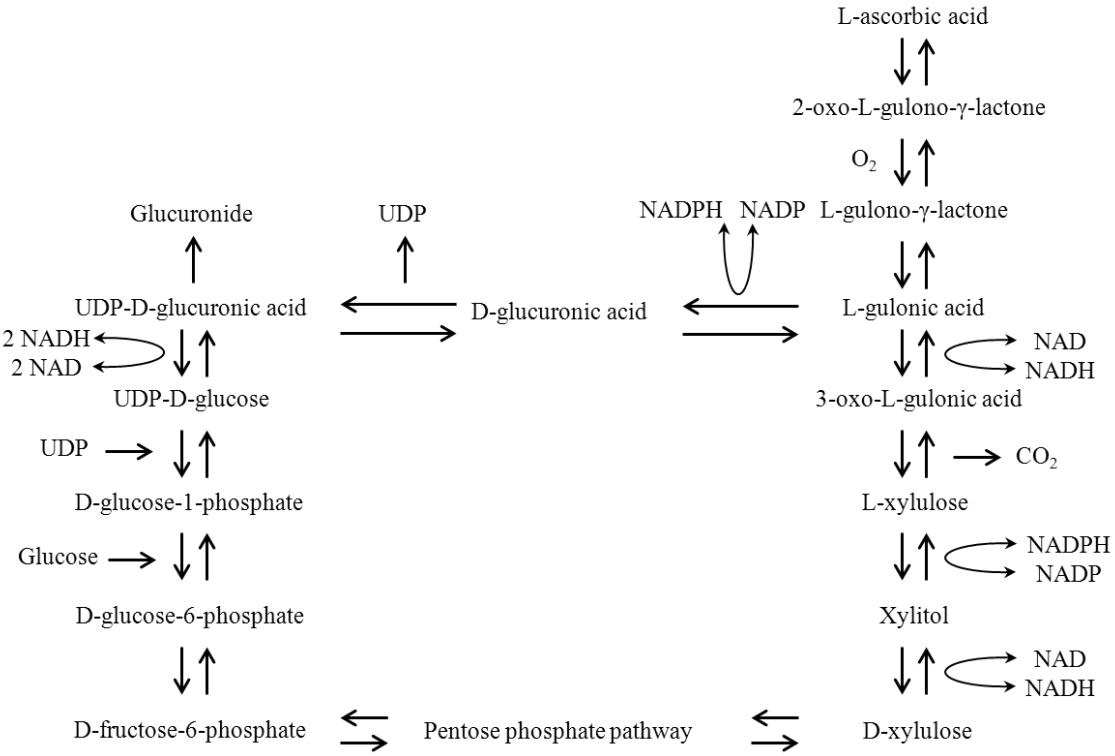

Figure 1. Glucuronic acid synthesis and metabolism in human liver.

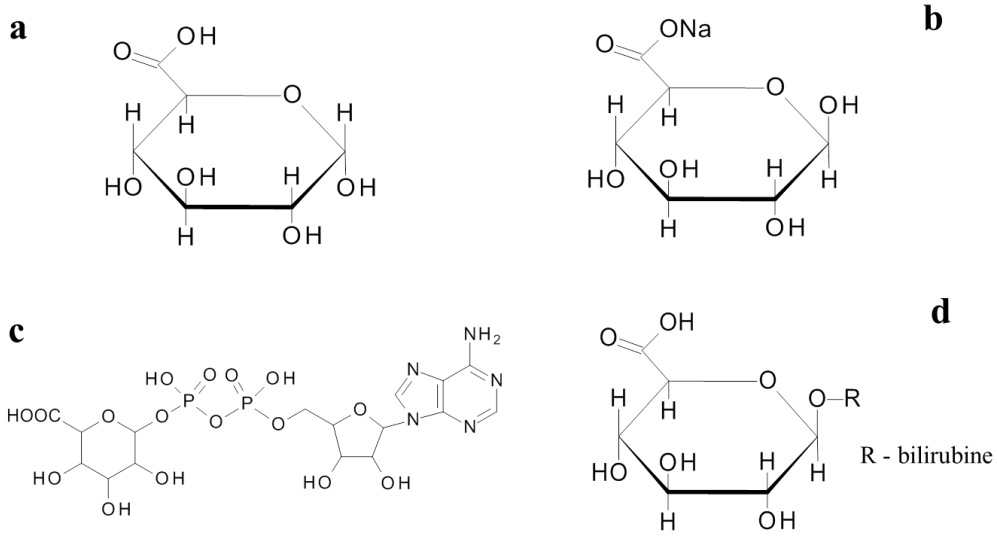

Figure 2. D-glucuronic acid and its metabolites:
a - D-glucuronic acid;
b - Na-D-glucuronate;
c - UDP-glucuronic acid;
d - bilirubin-glucuronide.

2.2. Metabolism of GlcUA in humans and in animals

The salts of GlcUA are known as glucuronates (Figure 2, b). The acid group of GlcUA, as it has been mentioned before, is ionised at pH 7, and so it usually exists *in vivo* as a glucuronate. GlcUA is also a component in nucleotide uridine diphosphate GlcUA (UDP-GlcUA, i.e., GlcUA linked to uridine diphosphate via a glycosidic bond), formed in the liver of all animals and humans (Figure 2, c). It is a cofactor for enzymes of glucuronidation's enzymes, called UGTs. As a result of glucuronidation the preformed GlcUA is utilized in the glucuronide formation (Figure 2, d). Many substances, including endogenous reactive metabolites, for example, bilirubin, and other potentially toxic endogenous and ingested substances, are excreted as glucuronides.

2.3. The variety of GlcUA functions in humans

Glucuronidation – one of the most important processes of detoxication. It has been proven that there are some differences in the capability of various species to biotransform separate exogenous chemicals and dietary components, for example, *in vitro* glucuronidation of dietary polyphenols in intestine and liver microsomes of rats or humans shows that even if the nature of the formed glucuronides is constant, the proportion of several metabolites varies widely, depending on the species and the organ [3-5]. Therefore, our focus is specifically on glucuronidation in humans. Xenobiotics - compounds alien for a normal biochemistry, are detoxified and excreted from the body, but many fat-soluble endobiotics, such as steroid hormones, fatty-soluble vitamins, essential unsaturated fatty acids, polyphenols of our diet and the others are activated and transported to target tissues via conjugation with GlcUA, that occurs mostly in the endoplasmic reticulum of liver cells according to the following general concept of detoxication (Figure 3).

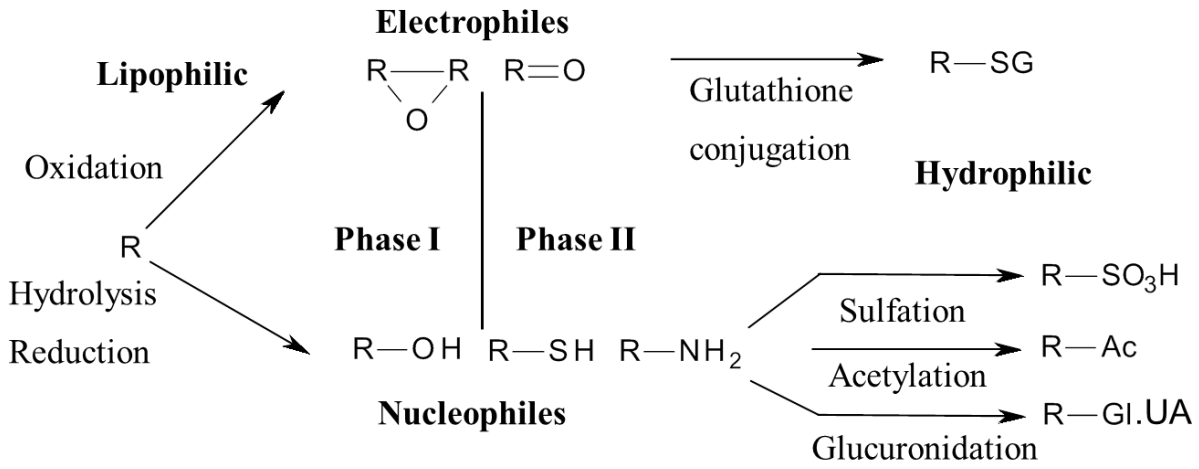

Figure 3. General concept of detoxication.

During Phase I reactions (Figure 3) various enzymes introduce small reactive polar groups containing positive and negative charges into the lipid-soluble molecule of toxicant or fat-soluble endobiotic. A lipophilic compound that has undergone a Phase I reaction is transformed to a new intermediate metabolite containing a reactive chemical group, e.g., hydroxyl (-OH), amino (-NH_2), carboxyl (-COOH) and S- containing.

Phase II reactions (Fig. 3) involve the addition of a small polar molecule to the GlcUA or another conjugating agent (it may or may not be preceded by a Phase I reaction). During Phase II reactions sulpfation (sulphur from methylsulfonylmethane and the other S-containing biologically active compounds), acetylation (N-acetyl-L-cysteine), amino acid (cysteine etc.), glutathione and GlcUA conjugation take place. GlcUA conjugation is the most common and important reaction of the Phase II. The substrates for glucuronidation are compounds that have oxygen, nitrogen, sulphur or carboxyl bonds, as aforementioned. Adequate amounts of GlcUA and the other conjugation agents are necessary for a proper detoxification capability. Conjugation makes xenobiotics and/or lipid-soluble endobiotics more water-soluble and delivers the glucuronides formed to an excretory system. The water-soluble conjugates can be transported and excreted by liver or by kidney, depending on the molar mass: those, having more higher molecular mass, are glucuronidated in the liver, transported in bile and excreted from the body via faeces; conjugates of smaller size are normally excreted via urine [6]. Guaranteeing of GlcUA in sufficient concentration in the human diet is important in relation to health maintenance and curative effects. Dynamic up-growth of GlcUA utilisation for detoxication of human organism nowadays (taking into account increased intake of xenobiotics) cannot be ensured only by the GlcUA synthesized in the liver; therefore, GlcUA can be derived from dietary sources - from the functional foods and/or beverages. Regular consumption of functional foods and drinks containing

GlcUA, for example, fermented beverages, obtained by using natural associations of bacteria and yeasts, e.g. Kombucha symbiosis, is the most simple and an effective remedy for human health protection. However, for such purposes the fermentation must be conducted by changing independent variables- the parameters of the process. The obtained final product can provide human requirements in GlcUA for detoxication and maintenance of normal metabolism of fat-soluble endobiotics.

2.4. Enzymes involved in the glucuronidation

Detoxification of xenobiotics - compounds, entering the body from the surrounding environment and toxic endobiotics, produced within the body, for example, bilirubin, oxidized fatty acids, are all expelled by an enzymatic pathway [7]. The binding of toxic compounds is realized by UGTs, a family of membrane-bound enzymes, which catalyse the transfer of GlcUA from UDP-GlcUA (an activated or coenzyme form of GlcUA) to xenobiotics, steroids and the other lipid-endobiotics, as well to polyphenols and other dietary components. Bio-transforming enzymes are widely distributed throughout the body, but the liver is the primary bio-transforming organ. The kidneys and lungs are next, with 10-30% of the liver's capacity. Low capacity exists in heart tissues, adrenal glands, the spleen, thymus, the skin, intestines, testes and the placenta of the human body. In the liver, the primary subcellular components that contain the transforming enzymes are the microsomes of endoplasmic reticulum and the soluble fraction of cytoplasm. Microsomal enzymes are highly associated with Phase I reactions, the most important being the cytochrome's P-450 enzyme system. Widely distributed among species, P-450 systems fall in two classes – bacterial/mitochondrial (Type I) and microsomal (Type II). NADPH, not NADH, is involved in the reactions of cytochrome P-450 [8]. A special feature of UGTs is a wide substrate specify; therefore, UGTs are involved in the biotransformation of the large number of compounds related to various chemical structures and origins. The information derived from the studies proposed by Radominska-Pandya offers an insight into the molecular mechanism of glucuronidation [9]. This information serves as a basis for the search for new drugs, for a better understanding of drug therapy, drug-drug interactions, and the risk of chemically induced diseases, including cancer [9]. Fifteen isoforms of UGTs have been identified in humans, having different tissue distribution [10]. The subfamily of UGTs known as UGT1A, for example, is localised in the intestine and include UGTs of polyphenols and bilirubin, identified as UGT1A1; the other isoform, UGT2A, glucuronidates the essential unsaturated fatty acids. Another enzyme glucuronidase works in the opposite way, which hydrolyses the glycosidic bond between GlcUA and other compounds and separates conjugated substances to free drugs, hormones and other lipid-soluble endobiotics in the target tissues. These enzymes are present in the lysosomes of various cells and can be released in response to oxidative stress in such pathologic cases as inflammations, cancer and AIDS [11-12]. The intestinal bacteria produce glucuronidase to break down glucuronides, thus allowing the reabsorption of GlcUA.

2.5. Health effects of glucuronidation

2.5.1. Glucuronidation for detoxication and elimination of xenobiotics

Xenobiotics can be classified as follows: drugs (antibiotics, antipyretics/analgesics, cardiac drugs etc.); carcinogens, as some food colorants, preservatives and artificial sweeteners; environmental chemicals; tobacco smoke toxins, nitrosamines, alcohol etc. There are many scientific reports on glucuronidation of xenobiotics in the human liver microsomes of endoplasmatic reticulum [13-16]. The metabolism of xenobiotics is usually divided into three phases – modification, conjugation and excretion.

Phase I reactions, i.e. modification. The cytochrome P-450 system is the most versatile biocatalyst, known to metabolise up to 50% of all drugs and other organic chemicals in phase I. In reactions of oxidation, reduction, hydrolysis and acetylation, the small polar groups containing positive and negative charges are joined to the lipophilic molecule of toxicant. Many of these intermediate metabolites do not possess sufficient hydrophilicity to permit elimination from the body. Therefore, these metabolites must undergo additional biotransformation via Phase II reactions.

Phase II reactions, i.e. conjugation. The conjugation of xenobiotics with hydrophilic molecular species such as GlcUA is known as Phase II metabolism [17]. The other Phase II reactions have been aforementioned (Figure 3). Glucuronidation or sulphation can often conjugate the same xenobiotics, but sulphation is a low-capacity pathway, for that reason glucuronidation is the most important reaction in the detoxication of xenobiotics, realized by UGTs of endoplasmatic reticulum and cytosol. These enzymes with a broad range of substrate specificity can metabolise almost any hydrophobic compound that has nucleophilic groups (Figure 3) [18-19]; glucuronidation and the other conjugations decrease the toxicity of xenobiotics; all conjugated metabolites are more water-soluble than the original xenobiotic or Phase I metabolites**.**

Phase III, i.e., excretion. The metabolites of Phase II usually are quite hydrophilic, therefore can diffuse across the membranes. Conjugates can be excreted from hepatic cells with the anionic groups aforementioned (Figure 3), acting as affinity tags for ATP-dependent membrane transporters- a huge variety of original hydrophobic

compounds occur [20]. So, Phase II products are removed to an extracellular medium and may be actively transported and excreted. If the GlcUA conjugated xenobiotic molar mass is large, therefore, excretion with bile becomes significant; smaller and highly polar sulphate conjugates are readily secreted in urine.

2.5.2. Detoxication and elimination of microbial toxins

The toxins produced by microorganisms are absorbed and conjugated with GlcUA, sulphate, glycine or glutathione in the aforementioned standard way. Recent studies have shown that plasma concentrations and urinary excretion of microbial toxins in humans is higher than those of tissular metabolites [21-23].

2.5.3. Glucuronidation for the elimination of bilirubin

Intermediate reactive metabolites are more polar and more water-soluble than the non-polar and lipid-soluble xenobiotics, although not all of them. Bilirubin (Figure 4, a) is insoluble in aqueous solutions, therefore, conjugation of bilirubin by albumin in the blood and by GlcUA in the liver is essential for its elimination. Bilirubin - the breakdown product of normal heme catabolism (haemoglobin from red blood cells) is the best example of a reactive metabolite, detoxicated and eliminated by glucuronidation. Bilirubin has different toxic effects: it uncouples oxidative phosphorylation, and as a result, inhibits activity of mitochondrial ATPase and action of a variety of enzymes from different classes, including dehydrogenases, hydrolases, enzymes of RNA, DNA, protein synthesis and carbohydrate metabolism. Bilirubin-UGT of hepatocytes conjugates 2 equivalents of GlcUA to produce bilirubin-diglucuronide (Figure 4, b). The increased water solubility of the tetrapyrrole facilitates its excretion as the bile pigment. According to the complexity of the tetrapyrrole chain and the large size of bilirubin diglucuronide it is excreted mostly in the bile moving out of the body through the digestive tract, where it is metabolized by colonic bacteria. A small amount of the conjugated bilirubin is eliminated by urine; elevated urinary bilirubin level is a general indicator of glucuronidation impairment.

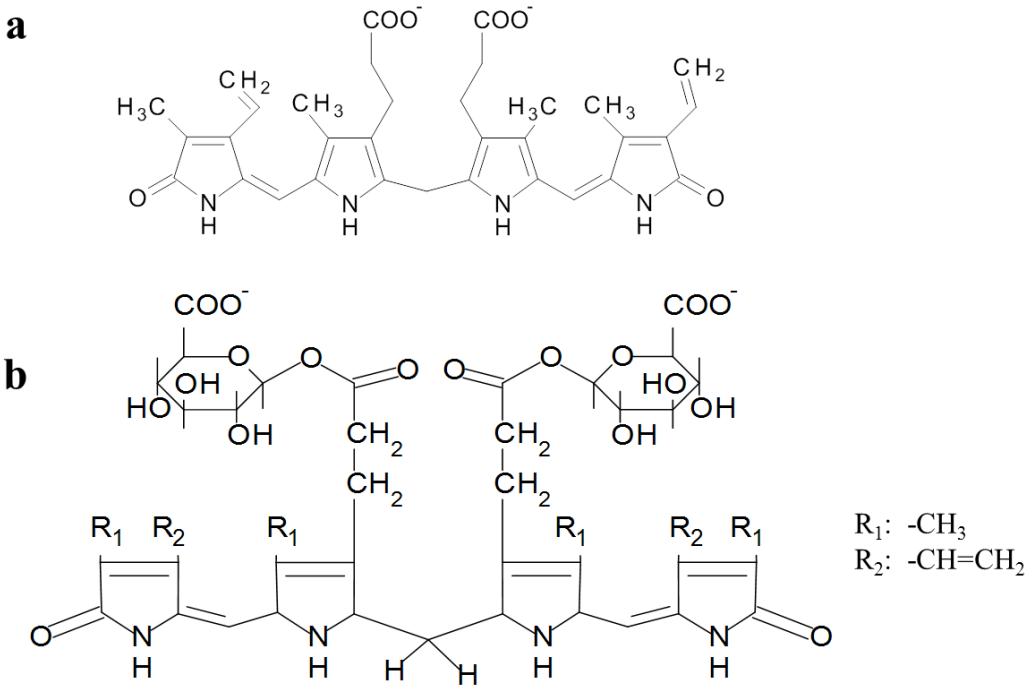

Figure 4. Glucuronidation of reactive metabolite bilirubine.

2.5.4. Glucuronidation for the improvement of bioavailability, i. e., bioactivation of polyphenols

Flavonoids ubiquitous in plants are an integral part of the human diet. The health effects of polyphenols depend on the amounts consumed and on their bioavailability [24]. Over the past decade, researchers and food manufacturers have become increasingly interested in polyphenols due to their potential antioxidant properties, great abundance in Western diet and preventive role as regards for chronic pathologies associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases [25-26]. Much attention has been focused on dietary phenolic antioxidants' effectiveness in protection against mutagenicity induced during lipid peroxidation, which are found to

inhibit the free-radical chain reaction of the cell membrane lipids; plant polyphenols have been mentioned as antitumor and antiviral agents [25]. Tea (*Camellia sinensis L.*) is one of the most popular beverages worldwide. Green tea (GT) is mainly consumed in Asian countries whereas black tea (BT) - in the Western nations. GT contains up to 30% of flavan-3-ols, commonly known as catechins. In the manufacturing of BT the monomeric flavan-3-ols undergo a polyphenol oxidase-dependent oxidative polymerization that leads to the production of theaflavins, thearubigins and other oligomers that give the characteristic colour and taste of BT. GT has been more effective than BT in the reduction of nitrosamine-induced tumour multiplicity (85% vs. 63%) and tumour incidence (30% vs. 7%); the oesophageal cancer incidence has been significantly reduced in all tea-treatment groups [25]. BT and GT, as principal components of Kombucha symbiosis fermentation media, is the general source of polyphenols for fermented beverages. Catechin and epicatechin from BT and GT are well known beneficial substances for human health prophylaxis [25, 27-30]. Catechins are shown to inhibit small intestinal, lung and mammary gland cancer genesis [25]; dietary flavonoids, quercetin and rutin have a considerable activity to suppress colon tumour incidence [25]. Quercetin is associated with a 23% reduced risk of pancreatic cancer [31]; it has anti-inflammatory properties [32-33]; reduces blood pressure and low density cholesterol (LDH) cholesterol levels in obese subjects [34-35] - the antioxidant potency of quercetin depends on the binding of the GlcUA. Many researchers have discussed the biological properties of the conjugated polyphenols according to their subsequent deconjugation and accumulation in the target tissues [4, 36-37]. Metabolism of polyphenols occurs via a common pathway - phenols are conjugated by the glucuronic and/or sulphuric acid [38-39]; that improves transport, bioavailability, and can affect the site of action and interactions of polyphenols with other antioxidants. As a result of glucuronidation, a remarkable bioactivation of ingested polyphenols takes place. UGT1A, localized in the tissues of intestine, plays a major role in the first-pass metabolism of polyphenols. These isoenzymes have a wide polymorphic expression pattern that could result in a high inter-individual variability in polyphenol glucuronidation. Polyphenols are secreted via the biliary route into the duodenum, where they are subjected to the action of bacterial enzymes, especially ß-glucuronidase, which promotes deglucuronidation - after that the polyphenols are reabsorbed. Such enterohepatic recycling may lead to a longer presence within the body; the concentration of polyphenols in the colon increases significantly [40].

2.5. Steroid hormones and fat-soluble vitamins D: increase in water-solubility, improvement of transport and bioavailability

Some sterols, for example, cholesterol and its derivatives - numerous steroid hormones and fat-soluble vitamins, in particular, calciferols-vitamins D, are essential for human health. The natural steroid hormones are lipids synthesized from cholesterol in the gonads and adrenal glands. A plenty of steroid hormones can be grouped in accordance to the receptors which they bind: estrogens, androgens, glucocorticoids, mineralocorticoids and progestins [41]. The deficiencies, as well the excesses of steroid hormones have an undesirable influence on human health. Glucuronidation can reduce both health dangers aforementioned: the first, i.e., deficiency by increasing the steroids' water solubility, by improving transport and bioavailability; the second, by facilitating the elimination of the excess steroids. The ability for glucuronides and sulphates of endogenous estrogens to exert biological activities after deconjugation at the cellular level has been observed [42-43].

2.6. The protective role of glucuronidation towards unsaturated fatty acids: increased water-solubility, improved interaction with polyphenols and other antioxidants

The insufficient transport and distribution of fat-soluble endobiotics throughout the body and the shortage of them in the targeted cells results in a more rapid progression of various NCDs.

Polyunsaturated fatty acids (PUFA), essential compounds of mammalian biomembranes, are very susceptible to peroxidation, which can ultimately breach membranes' integrity. The protection of living organisms against oxidative degradation is provided by antioxidants that reduce the rate of chain initiation and chain breaking, which interferes with one or more of the propagation steps- most phenols are chain-breaking antioxidants. Glucuronidation is an important pathway in the biotransformation and protection of fatty acids (FAs) - the energy sources, important structural components of cell membranes and the precursors of eicosanoids. FAs can also act as second messengers and regulators of signal transduction and, by those mechanisms, to play a significant role in controlling the growth, differentiation, proliferation and apoptosis of cells. It has been documented that in pathological conditions, oxidized fatty acids, including eicosanoids, are excreted in the form of glucuronides. Glucuronidation of FAs occurs in the endoplasmic reticulum and nuclear membranes of human tissues [44]. The research aforementioned helps to understand the detoxification of oxidized FAs and it can be used as important therapeutic strategy, such as the development of drugs targeting cardiovascular disease, inflammatory responses and cancer [44].

If the system lacks antioxidants, the reactive oxygen intermediates called the "free radicals", cause secondary oxidative damage to PUFA (omega-3 and omega-6) that can be oxidized, and lose their health benefits. It has been observed by Jayabalan, 2008, that glucuronidation, together with an increase in the antioxidant capacity of plasma

after the consumption of foods rich in polyphenols allow the Kombucha beverages to protect essential PUFA in human body; it has been proven that GT and BT have an inhibitory ratio against linoleic acid peroxidation of 38,46% and 34,4%, respectively [27].

3. GLcUA AS A STRUCTURAL COMPONENT OF ESSENTIAL ACIDIC MUCOPOLYSACCHARIDES

GlcUA is a constituent of various essential polysaccharides of the human body - acidic mucopolysaccharides, known as glycosaminoglycans (GAGs) and carbohydrate chains of proteoglycans, significant for preserving the structure-functional integrity in the organism. The most important GAGs are hyaluronic acid (HA), chondroitin sulphate (CS), heparin (H) and dermatan sulphate (DS). HA: the repeating disaccharide unit of HA comprises D-GlcUA and N-acetylglucosamine. Hyaluronic acid differs from other GAGs by a lack of sulphate groups and it is found not only in animal tissue, but in bacteria, as well. HA is a viscous jelly-like substance that fills the intercellular spaces of human tissues; it is present in the synovial fluid of joints, the vitreous humour of the eye, in the umbilical cord, in mucous secretions such as saliva and cell glycocalyx. The polysaccharide chain of HA is the longest of all GAGs, with a molar mass of $1x10^5$ to $1x10^7$ Da. High-molecular HA forms the structures of connective tissues and cartilage; hyaluronic acids of smaller molar mass are much less stiff and have excellent properties as lubricants. Chondroitin 4- and 6-sulphates: those are the most abundant GAGs in the body. CS is present in the cartilage, where it binds collagen and maintains fibres in a tight and strong network. Chondroitin contains D-GlcUA and N-acetylgalactosamine residues, sulphated on C-4 or C-6. Each chain may contain up to 100 individual sugars. CS is often marketed as a dietary supplement to prevent joint problems; it can also assist in liver functions. Heparin is known as a potent anticoagulant, used therapeutically to prevent clotting during intravenous therapy, as well as to inhibit clotting in various pathological conditions such as the period following a heart attack. H is built up by dimers of D-GlcUA and D-glucosamine; it is rich in sulphate groups - in average 2,5 sulphate groups have been found per a disaccharide unit. Unlike the other GAGs (that are extracellular compounds), heparin is an intercellular component; it inlays the cells of arteries, in particular, of liver and lungs. Dermatan sulphate originally is isolated from the skin, but it is found also in blood vessels and heart valves. The repeating disaccharide unit consists of iduronic acid (C-5 epimer of D-GlcUA) and N-acetylgalactosamine 4-sulphate. Iduronic acid is the predominant acid sugar, though a variable amount of β-D-GlcUA is also present. The description of the structure and functions of acidic mucopolysaccharides aforementioned, gives a positive statement on the GlcUA's necessity in a lot of essential functions in the human body.

4. FUNCTIONS OF GLcUA IN MICROORGANISMS

Various exopolysaccharides (EPS), including GlcUA containing, are synthesized by bacteria, for example, such EPS are characteristic for different strains of *Acetobacter* [45-47]. GlcUA is used as a carbon source in energetic metabolism. So, certain heterofermentative lactic acid bacteria, often encountered in fermentation of functional foods and beverages, are able to utilize GlcUA as the sole carbon source - almost 94% of the original glucuronate are converted to the final acidic products [48]. There is obvious evidence on the utilisation of GlcUA as an additional carbon source by *Gluconacetobacter* – the genus of bacteria often present in the Kombucha symbiosis. The ability of β-linked GlcUA oligomers (GAOs) to serve as a carbon source (alternative to glucose) has been assessed for *Gluconacetobacter hansenii* PJK - a producer of bacterial cellulose [49]. GlcUA is a constituent of bacterial cell wall. The outer membrane of a cell serves as a diffusion barrier for extracellular solutions. EPS produced by bacteria help to regulate an osmotic pressure inside the cell medium and in the fermentation medium as well. GlcUA is a component of capsules and slime layers, having multiple functions e. g. in pathogenic bacteria. GlcUA in the structure of hyaluronic acid of the pathogenic bacteria capsules contributes to the invasiveness of pathogens. GlcUA is a precursor of L-ascorbic acid (vitamin C) synthesis in Kombucha beverages [50-52]. The synthesis represents a "side arm" of the GlcUA pathway, branching off from the L-gulonic acid (Figure 1). The functions of GlcUA in the microorganisms aforementioned only partly describe the variety of them; bacteria and yeasts utilize GlcUA for a number of essential requirements.

4.1. Synthesis of GlcUA by natural association of bacteria and yeasts

A natural association of bacteria and yeasts, called the "Kombucha", has been studied by many researchers [53-54]. Acetic acid bacteria found in the Kombucha association are *Acetobacter xylinum* [55], *Acetobacter xylinoides, Bacterium gluconicum* [56], *Acetobacter aceti, Acetobacter pasteurianus* [57] and *Gluconobacter oxydans* [58-59]. The yeast species identified in Kombucha symbiosis are: *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Torulaspora delbrueckii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii, Candida stellata, Torulopsis sp., Pichia sp.* [55, 57-61]. Two strains unique for the Kombucha symbiosis - bacteria *Gluconacetobacter kombucha sp.* [62] and ascosporogenous yeast *Zygosaccharomyces kombuchaensis*, have been identified in a beverage fermented

by Kombucha [59, 63]. It is noted, that the composition of Kombucha symbiosis is highly variable [58]. In correspondence, the composition of active compounds in functional beverages greatly depends upon the individual association of microorganisms being used. Basic biochemistry of the Kombucha action remains largely unknown [60]. The present review overviews a possibility to obtain increased yields of GlcUA by changing the conditions of the Kombucha fermentation. Different symbiotic associations of acetic acid bacteria and yeasts have been cultivated by the researchers (Table 1, column 3). All the Kombucha symbiosis being used have produced GlcUA, and the data obtained demonstrate that the fermentation can be conducted as it has been mentioned previously [64-70]. The increasing quantities of GlcUA obtained are demonstrated in the seven examples, arranged in order of the increasing yields (Table 1, column 9). The optimal independent variables of the process for increasing the yield of GlcUA are described in columns 4-8. To simplify the understanding of the examples reported, a brief description of the main variables of the Kombucha fermentation has been done**.** It is emphasized that the synthesis of Kombucha products is determined by the following fermentation conditions: sugar and tea being used, the temperature of the fermentation and duration of the process etc.

Table 1. Conducted fermentation of Kombucha to enhance GlcUA yield.

			Independent variables of fermentation process					
No	Origin of Kombucha	Composition of Kombucha symbiosis (bacteria & yeasts)	Carbon & energy source - sugar substrate, g/L	Nitrogen and growth factor source - Tea: black, green, herbal; atypical substrates	t^0 temperature ^oC	T (time) - duration the of process, days	Yield of GlcUA mg/ml; g/L	References
1.	2.	3.	4.	5.	6.	7.	9.	10.
1.	France, Tunisia	- *Acetobacter xylinum* - *Zygosaccharomyces rouxii*, - *Candida sp.*	Sucrose	BT	-	7	<10 mg/L	[64]
2.	Serbia	- *Acetobacter xylinum*, - *Saccharomycodes ludwigii* - *Saccharomyces cerevisiae* - *Torulopsis sp.* - *Zygosaccharomyces sp.*	Sucrose 100 g/L	BT	28	21	3,39 mg/L	[65]
3.	Iran	- *Acetobacter xylinum* - *Brettanomyces*, - *Zygosaccharomyces*, - *Saccharomyces*, - *Pichia*	Sucrose; Corn syrup, molasses	BT	31	7	44,5 mg/L	[66]
4.	India, Coimbatore Tamil Nadu	Kombucha mat (local)	Sucrose 100 g/L	GT BT	24±3	18	1,73±0,14 g/L GT 2,33±0,24 g/L BT	[67]
5.	Korea	*Gluconacetobacter hansenii* PJK	Total carbohydrates 29,44 g/L	Waste beer fermentation broth	30	10	112,65 g/L	[68]
6.	Iran (Persian Type culture collection IROST)	- *Acetobacter xylinum* - *Brettanomyces*, - *Zygosaccharomyces*, - *Saccharomyces*, - *Pichia*	Sucrose, 10 g/L	Sour Cherry Juice Inoculum: BT	37	14	132,5 g/L	[69]
7.	Iran (Persian Type culture collection IROST)	- *Acetobacter xylinum* - *Brettanomyces*, - *Zygosaccharomyces*, - *Saccharomyces*, - *Pichia*	Sucrose, 9 g/L	Grape Juice Inoculum: BT	37 18 27 37	14	178,0 g/L	[70]

GlcUA is produced when glucose-containing sugars, such as sucrose (glucose, fructose), maltose (glucose, glucose), lactose (glucose, galactose) etc. are used in a sufficient quantity. Sucrose, as a source of carbon, is hydrolyzed enzymatically to free-fructose and glucose. Fructose serves as an energy source for bacterial growth; glucose, mainly, is the substrate for synthesis of the final products. Plenty of different compounds, significant for human health protection, have been produced during the fermentation of the Kombucha symbiosis. A wide range of organic acids, such as GlcUA, gluconic acid, lactic, acetic, succinic, butyric, malic and usnic acid; vitamins of group B, and vitamin C in particular, free amino acids and different active enzymes are present in the final beverage. They together may realize significant health effects in spite of small concentrations of active compounds by acting synergetically. GlcUA is considered to be one of the key components found in fermented beverages due to its detoxifying action.

4.2. Fermentation media

Two different versions of Kombucha symbiosis inoculums have been used - the cellulose containing coherent top layer or a culture liquid from previous fermentation [71]. The main independent variables of the Kombucha fermentation process are the carbon and nitrogen (and growth factor) source, i.e., sugar and tea substrates, or some atypical fermentation media - herbal teas, juices of fruits and berries; the temperature and duration of the fermentation process, while the response variables are the growth of biomass and yield of target compound, for example, GlcUA, remained sucrose, total acidity and pH.

The traditional carbon source for Kombucha fermentation is sucrose [72]. A number of investigations have been reported about conversion of sucrose into various final compounds [56, 63, 73-74]. Fermentation of Kombucha association initial has been made by using 10% sucrose-sweetened BT or GT - the usual classic substrates for the preparation of Kombucha beverages [27, 56, 75]. Further, the researchers consider that a higher concentration of sugar is optimal for the growth of the Kombucha symbionts and synthesis of the final products, including GlcUA [56, 64, 72, 76], 20% sucrose has been used to intensify the fermentation process [77]. The final results depend on the blend of the tea used. The researchers have carried out systematic studies on a broad concentration range (1,25 g/L - 12,5 g/L w/v) of tea substrate; it turned out, that symbionts have been able to synthesize GlcUA, only the yield of it has been low [65, 78-81]. Tea leaves are rich in polyphenols, known as effective antioxidants; some other metabolites of brewed drinks (vitamin B_2, C and organic acids) also can act as antioxidants [82]. It has been observed that the protective nutrients of tea, having antioxidant properties, stimulate the microorganisms' growth and increase the yield of GlcUA in a shorter time [27, 58, 67, 83]. The search for a fermentation medium rich in antioxidants has led to a new direction of the research - the fermentation of Kombucha on herbal teas used in traditional medicine [77, 82, 84, 85] and on other dietary substrates, to enrich these products by natural compounds of microbial origin that are beneficial for human health. There are studies on fermented milk [71, 72, 86, 87], beer, red and/or white wine, cheese whey [76,79] and on agricultural and industrial by-products, such as molasses from sugar-beet processing [80]. A special subject is applying the juices of some fruits and berries containing the potent antioxidants as a fermentation media [69, 70, 88].

5. GlcUA: UTILIZATION OF GLcUA AND ITS DERIVATIVES IN MEDICINE

Polysaccharides containing GlcUA and its derivatives possess structural and functional diversity and have wide application in medicine.

A: GlcUA and its oligomers (GAOs) are the components of pharmaceuticals and biocomposites, for example, HA has become a widely utilized substance among plastic and reconstructive surgeons as a hypoallergenic surgical material for reconstructing and building up tissues lost. GAOs, due to their high water-solubility and repeating carboxyl groups, are valuable solubility enhancers for lipophilic or high-polymeric substances in water medium, and as carriers and stabilizers for various active substances (i.e., for the immobilization of enzymes) [89]. Biological activities of low-molecular weight glucuronans on mammalian cells have been confirmed recently, with a claim describing stimulation of elasticity of the dermis and epidermis by acetylated oligoglucuronans with degrees of polymerisation of 18-19 [90]. GAOs are indicated in infectious and inflammatory diseases, transplantation, neutralization of toxins, cancer immunotherapy, and metabolic and cardiovascular diseases, and in pharmacy for drug delivery [91-92].

B: GlcUA and its oligomers are used as pharmaceuticals, for example, HA serves as a lubricant and shock absorber; heparin is used as an anticoagulant. GlcUA in the human body can be converted into chondroitin-sulphate associated with cartilage, collagen and the fluid that lubricates joints [93].

C: GlcUA is used for medical research, for example, for the determination of urinary steroids and steroid conjugates in the blood.

Kombucha beverage health effects: Fermented beverages, containing GlcUA, e. g., Kombucha, may promote immunity and general well-being. Immunostimulating activities on human blood monocytes are also described, as

low molecular weight glucuronans induced the production of cytokines IL-1, IL-6 and TNF-α [94]. Particular attention is given to Kombucha beverages due to their energizing properties and beneficial influence on the digestive system [73]. Most often the utilization of beverages fermented by Kombucha are focused on the supposed detoxifying effects, determined by high content of GlcUA. It is considered that the detoxifying property of a Kombucha beverage is presumably due to the capacity of GlcUA to bind the molecules of toxins and the increased elimination of them by the kidneys or the intestines [67]. The health-beneficial effects of Kombucha beverages are due to the presence of different vitamins of group B, vitamin C; antibacterial properties, are due to the presence of usnic acid and the other antimicrobial components, acting synergetically [64, 95-97]. Many diseases can impair an individual's capacity to biotransform xenobiotics. A good example is hepatitis: it is shown, that Kombucha reduces hepatic detoxication to less than half of the normal capacity and prevents the paracetamol-induced hepatotoxicity [98]. Regular Kombucha beverage ingestion contributes significantly to weight gain inhibition and prolongation of the life span [99]. There are enumerated health effects, such as reducing the blood pressure, the possibility to relieve arthritis, to increase immune response and to cure chronic illnesses, caused by oxidative stress, like cardio-vascular, neurodegenerative, for example, Parkinson's disease and cancer [27]. The GlcUA is found to act also as an antioxidant in humans [100]. Oral administration of a Kombucha-fermented beverage to rats exposed to pro-oxidation have been indicated the potent antioxidant properties of the fermented drinks, such as decrease of the degree of lipid oxidation [101-102]. Unbalanced oxidative stress is known as an inducer of various diseases [103]. Antiproliferative activity of BT, Kombucha, and *Satureja montana* Kombucha beverages has been studied on different kinds of cancer cells lines: HeLa (cervix epitheloid carcinoma), HT-29 (colon adenocarcinoma), and MCF-7 (breast adenocarcinoma) [104]. The effects of feeding a Kombucha beverage to a duck indicated that this fermented drink decreases levels of total cholesterol and LDH and, simultaneously, increases level of high density cholesterol (HDH) in blood: it means that the high content of GlcUA in a Kombucha beverage can neutralize the cholesterol deposits in human body by changing them to a more water-soluble compound for enhanced transporting and elimination [105].

6. CONCLUSIONS

The present review extends a common notion about the role of the glucuronidation in humans for normal functioning of organism and health protection: a detailed exposition of the general concept and the biochemical mechanism of glucuronidation is reported; the present review provides the interpretation on the role of glucuronidation for bioactivation of essential fat-soluble endobiotics and detoxication of xenobiotics. The hypothesis about the possible synthesis of GlcUA by natural associations of bacteria and yeasts has been confirmed. The experimental studies overviewed a point toward the possibility to increase the yield of GlcUA in functional beverages of Kombucha by a purposeful replacement of the fermentation medium and changing of process-independent variables: a high concentration of sucrose, increase of temperature and a fermentation medium rich in antioxidants have the causative role on improvement of the microorganisms' cell growth and production of GlcUA.
Regular guaranteeing of human body with a sufficient quantity of GlcUA is important in respect to health maintenance and different curative effects, therefore, the consumption of Kombucha beverages, containing GlcUA is recommendable for detoxication and elimination of xenobiotics, support of fat–soluble endobiotics' normal metabolism and prevention of NCDs.

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