# Comparative Study on the Effect of Sweeteners on the Oxidative Status of Green Tea and Black Tea

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Both green and black tea are known by the consumers for their chemical composition rich in polyphenols – substances with an important antioxidant effect, proofing the capacity to fight against free radicals and to strengthen the immunity system. In order to improve the sensorial characteristics of green and black tea, the main food additives used are natural and synthetic sweeteners. Knowing how to choose the best sweetener for organic green and black tea is a duty of any specialist in this field, in order to improve the capacity to make nutritional recommendations – taking into consideration the different health level of the consumers (especially the ones with diabetes or cardiovascular symptoms or diseases, digestive or hormonal problems or allergies). The present paper shows a personal method for finding of the best synthetic or natural sweeteners that can be used for sweetening the organic green or black tea coming from areas without any contamination. This method allows the selection of optimal sweetener by monitoring the oxidative status of internal and external environment for green and black tea.

Keywords: green tea, black tea, sweeteners, coenzymes (NAD, FMN)

The tea is most widely consumed beverage in the world, next to water. In U.S.A. (bigger consumption in world) the tea consumption is grouped in black tea (85%), green tea (14%) and others (1%). Today, around the world, 15 000 cups of tea are consumed in every second; black tea is the favourite tea assortment - 78% of world consumption, at the same time, green tea is preferred by 20% of world consumers [1]. Standard amount of caffeine in a cup of green tea is around 35-80mg/100 g (one cup), in black tea is around 90-110 mg/100g; in average cup of coffee will get about 110-130 mg/100 g caffeine [2].

get about 110-130 mg/100 g caffeine [2]. Green tea is a type of tea obtained from leaves unfermented by *Camelia sinensis*. In this type of tea, the post-harvest natural fermentation process is stopped (a major difference from black tea). Green tea differs from the black one by way of preparation, content of substances, taste and produced effects.

The used technologies in tea industry are shown in figure 1 [3].

Both, in green tea and black tea will be comparable quantities of epicatechin, catechin, and epicatechingallate. In addition, in the green tea will be larger amounts of gallic acid, gallocatechin gallate, kaempferol, myricetin, quercetin, and routine - than in black tea [4]. Green tea contains several catechins, but the polyphenol with the highest anticancer activity of all is EGCG or gallate epigallocatechin [5]. Polyphenols of green and black tea attenuate blood pressure increases due to their antioxidant properties [6].

The principal reasons for should drink more green tea can be grouped: the teas of all varieties contains high levels of antioxidant (polyphenols), drinking tea in place of light calorie beverages can help people lose weight, despite the caffeine, green tea can help keep people hydrated (through hydrating the body) [7]. Recently, some epidemiological studies have indicated that drinking green tea slightly reduces blood pressure. 120 mL of green tea consumed per day for 1 year significantly reduces the risk of developing hypertension [8]. Black tea can also expand the airways and making breathing easier for asthmatics [9]. To improve sensory characteristics of green tea and black tea are commonly used natural sweeteners or synthetic sweeteners and these must be chosen carefully because using them singly or together with other food additives can lead to severe illness [10].

The green tea and black tea are different limits of maximum admissible concentrations for certain metals in green and black teas from China, India, Pakistan, Brazil (different limits according to the legislation of these tea producing countries) [11].

To highlight the occurring changes in the oxidative status of green and black teas, to determine how the antioxidant potential of these can improve the nutritional characteristics



Fig. 1. The used technologies in tea industry (Typical sequences of green and black tea manufacturing in South-East of Asia) [3].

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of refreshing and energizing drinks (following the addition of natural or synthetic sweeteners), it is normal to know the changes in the oxidized and reduced concentrations of the main specific coenzymes (NAD and FMN) of oxidoreductases – which act on interior or on exterior of these teas. These changes in concentrations (both for oxidized and reduced forms of NAD and FMN) are specific indicators of final teas - indicators that show us the best consumption variants and possible consumer-induced effects [12].

The coenzyme Nicotinamide Adenine Dinucleotide (NAD) is a dinucleotide, because it consists of two nucleotides joined through their phosphate groups [13]. One nucleotide contains an adenine base and the other, nicotinamide. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as NAD<sup>+</sup> and NADH respectively [14]

The coenzyme is, therefore, found in two forms in cells: NAD<sup>+</sup> is an oxidizing agent – it accepts electrons from other molecules and becomes reduced [15]. This reaction forms NADH, which can then be used as a reducing agent to donate electrons [16]. These electron transfer reactions are the main function of NAD. In metabolism, the compound accepts or donates electrons in many redox reactions [17].

Because of the importance of these functions, the enzymes involved in NAD metabolism are targets for functional food and drug discovery [18]. The main role of NAD<sup>+</sup> in metabolism is the transfer of electrons from one molecule to another.

Both NAD<sup>+</sup> and NADH strongly absorb ultraviolet light because of the adenine. From literature, the peak at absorption of NAD+ is a wavelength of 259 nanometres (nm), with an extinction coefficient of 16,900 M<sup>-1</sup>cm<sup>-1</sup> [20]. NADH also absorbs at higher wavelengths, with a second peak in UV absorption at 339 nm with an extinction coefficient of 6,220 M<sup>-1</sup>cm<sup>-1</sup> [19]. This difference in the ultraviolet absorption spectra between the oxidized and reduced forms of the coenzymes at higher wavelengths makes it simple to measure the conversion of one to another in enzyme assays - by measuring the amount of UV absorption at 340 nm using a spectrophotometer [20]. This energy is transferred to NAD<sup>+</sup> by reduction to NADH, as part of beta oxidation, glycolysis, and others biochemical cycle [21].

These oxidoreductases are very active in foods that are rich in antioxidant – like as green tea and less in black tea

[22]. A similar behaviour has been studied in an applied research on the metabolism of other medicinal herbs [23].

The oxidoreductases activity may be influenced by the active substances of sweeteners that are able to develop some fields of different energies. These energy fields are more intense when using naturally sweeteners with high caloric energy. The oxidoreductases activity may be influenced by the ions from water which are used in extract or solution [24].

It is therefore very important the study of sweeteners action in green tea and black tea through analysing the concentrations of reduced and oxidized forms of NAD and FMN (to know the effects on aerobic and anaerobic environment of teas).

Since both the oxidized and reduced forms of nicotinamide adenine dinucleotide are used in main sets of reactions, the cell maintains significant concentrations of both NAD<sup>+</sup> and NADH, with the high NAD<sup>+</sup>/NADH ratio allowing this coenzyme to act as both an oxidizing and a reducing agent [25].

Flavin mononucleotide (FMN), or riboflavin-52 phosphate, is a bio-molecule produced from riboflavin (vitamin B<sub>2</sub>) by the enzyme riboflavin kinase and functions as prosthetic group of various oxidoreductases including NADH dehydrogenase as well as cofactor in biological blue-light photo receptors. During the catalytic cycle, a reversible interconversion of the oxidized (FMN), semiquinone (FMNH<sup>•</sup>) and reduced (FMNH<sub>2</sub>) forms occurs in the various oxidoreductases [26]. FMN is a stronger oxidizing agent than NAD and is particularly useful because it can take part in both oneand two-electron transfers. In its role as blue-light photo receptor, (oxidized) FMN stands out from the 'conventional' photo receptors as the signalling state and not an E/Z isomerisation [27]. Flavin mononucleotide is a coenzyme for a number of oxidative enzymes including NADH Dehydrogenase. It is the principal form in which riboflavin is found in cells and tissues.

# **Experimental part**

#### Microwave digestion -Method

In order to determine correctly the influence of certain natural and synthetic sweeteners on the level of oxidative status green tea and for sample preparation were performed chemical analysis for the determination of heavy metals and minerals from several varieties of green teas and black teas. It is very important that the final food (the sweetened green and black teas) to retain the

Power ,W	Time, min	Agitation	Comment
300	5	Yes	For protect of cartridges
0	2	No	For helping the sedimentation process in to blase of cartridge
400	3	Yes	
600	3	Yes	
200	1	No	
800	3	Yes	
1000	5	Yes	
0	10	No	Cooling for open the cartridges

Table 1THE MICROWAVE DIGESTION STEPS

characteristics of the primary functional food (with very high antioxidant potential) and be non-toxic to the consumer. Therefore, were checked (before the experiment) several kinds of green and black teas and analysed by atomic absorption spectrometry the concentrations of certain chemical elements (heavy metals) - to avoid the use of these teas with traces of pollution.

For experiment were used green tea and black tea plants which showed no traces of pollution or contamination and therefore could not generated pre-catalytic oxidation conditions.

In the first phase sample preparation for atomic absorption spectrometry was used mineralization (microwave digestion). Microwave digestion is used to prepare samples of all types (plant, soil, food, pharmaceuticals) for elemental analysis by ICP, ICP-MS, or AA, which require the sample to be in the form of a solution in order to introduce it into the analyser. Acid digestion is employed to break down the sample matrix leaving the elements of interest in solution and ready for analysis. CEM microwave digestion systems rapidly break down a wide variety of sample matrices leaving behind a clear solution containing the analytes of interest.

Microwave technology has become a common tool for chemical synthesis both in academia laboratories and industry. Compared to conventional ways of synthesis, the advantages of heating with a microwave include: faster reaction times, better reproducibility, improved purity, greater yields and enhanced management control.

The microwave accelerated reaction system is designed for digesting, dissolving, hydrolysing a wide variety of materials in a laboratory setting. The system uses microwave energy to heat samples in polar or ionic solutions rapidly and at elevated pressures. Its main purpose is for preparing samples for analysis by atomic absorption (AA)

For this purpose it used a CEM Mars system of microwave mineralized, 1200W. The MARS system of CEM is a multimode platform equipped with a magnetic stirring plate and a rotor that allows the parallel processing of several vessels per batch. We used the HP-500 (Teflon (TFA) insert) (vessel volume 80 mL, max pressure 350 psi, max temperature 210°C) and Greenchem (glass (borosilicate) insert) (vessel volume 80 mL, max pressure 200 psi, max temperature 200°C) vessel assembly types both based on a fourteen positions rotor. The system delivers a continuous power output between 0 and 1200 W. Temperature is controlled internally by fibber optic probe in one control reference vessel. On-line pressure monitoring of the reference vessel is also provided. All rotor segments are protected by a vent nut that contains a rupture membrane. Additionally, the system is equipped with a solvent sensor detector safety feature. Cooling down of the rotor segments to room temperature is done by an air flow provided by the exhaust fan.

Method: Briefly, was weighed with analytical precision 10 g dry substance (d.s.). For the mineralization was added to each digestion cartridge 10.0000 g product (green tea one variant and separately, on other step, black tea one variant), 6 mL of concentrated nitric acid and 3 mL of 30% hydrogen peroxide. For blank was used one digestion cartridge without product, just reagents. The method is summarized in table 1.

## Atomic Absorption and UV Vis Spectroscopy

For AAS method was used one Varian SpectrAA 220Z Atomic Absorption Spectrometer Furnace System with Varian SpectrÂA 220Ź Auto Sampler, Varian GTA 110Z 1408

Furnace, Varian UltrAA and afferent Windows interface software.

For to obtain of the witness experimental variant of green tea (unsweetened-V1 GT Mt) it has been used green tea packed in little special envelopes and that was boiled and adequate separated.

For to obtain of the witness experimental variant of black tea (unsweetened- V1a BT Mt) it has been used black tea packed in little special envelopes and that was boiled and adequate separated.

One green tea packed in a 100 g bag has been used as basis for experiment. It was used a tablespoon of green tea in 250 mL water (at 70°C) in a steady 3 min infusion. This variant was the basis for experimental variants  $V_1$ - $V_{s}$ .

One black tea packed in a 100 g bag has been used as basis for experimental variants  $V_{1a}$ - $V_{8a}$ . It was used a tablespoon of black tea in 250 mL water (at 70°C) in a steady 3 minutes infusion.

The sweetening operation was carried out under the same conditions of temperature and pressure. To avoid errors of analysis were verified areas of maximum absorbance (where molecular absorption spectra recorded a maximum peak) for NAD, NADH + H<sup>+</sup>, FMN; FMNH + H<sup>+</sup> and used the Unique Addition method and standards Pure Analysis substances for each compound using as the baseline unsweetened green tea (for experimental variants  $V_1$ - $V_3$ ), respectively, black tea (for experimental variants  $V_1^{-}$ - $V_{8a}$ ). In the Unique Addition method were used NAD Pure

Analysis substance (20mg/vial type N8410-15VL, stored at -20°C) and Flavin Mononucleotide FMN Pure Analysis (100 mg/pack tip CDS020791).  $\beta$ -Nicotinamide adenine dinucleotide (NAD+) and  $\beta$ -Nicotinamide adenine dinucleotide, reduced (NADH) comprise a coenzyme redox pair (NAD+: NADH) involved in a wide range of enzyme catalysed oxidation reduction reactions.

In order to achieve a correct correlation have been measured the variation of pH and redox potential to experimental variants – follow the applied sweetening operation. Both results achieved by UV-Vis spectral analysis and the results of the variation in pH and Eh (redox potential) were subjected to statistical analysis mathematical correct interpretation of the results.

For experimental variants  $V_1$ - $V_8$ After being boiled (50-60g green tea / L water) and cooled, the obtained drink was decanted and filtered (through a porous cellulosic material). After filtration task, the green tea was centrifugal separated into a performance centrifuge *Sygma* type, at a 4400 rot/min during 10 minutes.

After the centrifugal separation had been picked a median sample of 50 mL green tea drink that was diluted (1:10 with ultrapure water) ; this variant being the unsweetened reference one ( $V_1$ -GT Mt).

At this reference sample it had been added the principal sweeteners admitted in Romania: naturals or syntheticsobtaining other 7 experimental variants:

 $V_1$ - unsweetened green tea (GT = reference sample);  $V_2$ - green tea with sugar sweetener (GT + sugar);  $V_3$ - green tea with saccharine sweetener (GT +

saccharine):

V<sub>4</sub>- green tea with Aspartame sweetener (from "Equal" product) (GT +Aspartame);

 $V_r$ - green tea with Edulciclam sweetener (GT + Edulciclam);

 $V_6$  - green tea with Zuckli sweetener (GT + Zuckli);  $V_6$  - green tea with Sucrazit sweetener (CT - Sucrazit

- green tea with Sucrazit sweetener (GT + Sucrazit);

 $V_{s}$  - green tea with honey (GT + Honey).

For experimental variants  $V_{12}$ - $V_{23}$ 

After being boiled (50-60g black tea / L water) and cooled, the obtained drink was decanted and filtered (through a highly porous cellulosic material). After filtration task, the black tea was centrifugal separated into a performance centrifuge Sygma type, at a 4800 rot/min during 10 min.

After the centrifugal separation had been picked a median sample of 50 mL green tea drink that was diluted (1:20 with ultrapure water) ; this variant being the unsweetened reference one ( $V_1$ -BT Mt). At this reference sample it had been added the principal

sweeteners admitted in Romania: naturals or syntheticsobtaining other 7 experimental variants:

 $V_{1a}$  unsweetened black tea (BT = reference sample);

 $V_{2a}^{1a}$ - black tea with sugar sweetener (BT + sugar);  $V_{3a}^{2a}$ - black tea with saccharine sweetener (BT + saccharine);

V<sub>42</sub>- black tea with Aspartame sweetener (from equal product) (BT +Aspartame);

V.- black tea with Edulciclam sweetener (BT + Edulciclam);

 $V_{ba}^{a}$  - black tea with Zuckli sweetener (BT + Zuckli);

 $V_{7a}^{ua}$  - black tea with Sucrazit sweetener (BT + Sucrazit);  $V_{7a}^{a}$  - black tea with boney (BT - U

<sup>\*</sup> - black tea with honey (BT + Honey).

The saccharine had 19 mg saccharine/tablet had been added into boiled tea; the Aspartame 90 mg Equal/tablet, Edulciclame 100 mg Natrium Cyclamate/tablet Zuckli 40 mg Cyclamate + 4mg Saccharine/ tablet, Sucrazit (Natrium bicarbonate 59.52%, Saccharine 23.81%, fumaric acid 16.67% for one tablet were too added into boiled tea. The used honey was obtained from Acacia flowers.

The experimental samples were spectrophotometer to a digital high performance spectrophotometer UV-Vis T92+ type from PG Instruments, in the nearly UV range (190-400 nm), the visible range (400-700 nm) and nearly IR range (700-900 nm). The T92+ is a high performance double beam spectrophotometer with a variable spectral bandwidth from 0.1-5nm, selected by a continuous variable slit and Photometric Range -4.0 to 4.0Abs and Photometric Mode: Transmission, Absorption, Reflectance, and Energy & Concentration. At 325 nm was automat interchanged the Deuterium lamp with a Wolfram one (was selectable within the working range of light source).

For minimise analytical errors it used a thermostatic system controlled in all mean UV-Vis-IR controlled by a manual re-tracking (at accuracy of ±0.3nm (Automatic Wavelength Correction). During the analysis for the experimental variants it has been taken all the treatments, for having a minimal temperature changes at the maxim limit of the interfering substances influence, the assure the optimal needed conditions for an average analytical errors limits. During the analysis and the interpretation of the results it has been used from the utilitarian packet MS Office 2000: MS Word2000 and MS Excel 2000.

The changes induced by sweeteners on the antioxidant potential of experimental variants of green tea and black tea can be recorded by following the concentrations of the oxidized forms and the reduced forms of the major oxidoreductases enzymes which activate in these teas.

These oxidoreductases are capable of managing and monitoring - through the mechanisms and the field of action induced in the reaction medium - the main redox processes that occur in green and black teas. The redox processes that occur in the green tea and black tea medium are modified as a result of the addition of permitted sweeteners (to improve the sensory characteristics of teas) and as well as mass, heat and impulse transfer phenomena.

#### **Results and discussions**

The results in determining the heavy metal and mineral elements contain from both - green tea and black tea used for check of sweetening effect on NAD and FMN coenzymes are represented in the table number 2.

In order to observe how high element content is in the tea type chosen for sweeting test, these elements were quantified also from the chemical tries on aqueous extract (1:10 diluted with ultrapure water for green tea and 1:20 diluted with ultrapure water for black tea).

It has been controlled experimentally the influence of the sweeteners added in the green tea drink, evident the Absorption in the nearly UV range, Vis and near IR rage too.

After the process of spectrophotometry on UV-Vis-IR ranges, it had been obtaining more than 1100 pairs of data.

From the changes in concentrations of NAD analysis, it can be seen that in an anaerobic environment were recorded the smallest concentrations of oxidized

Indicator/ Constituent	Green Tea		Black Tea	
	Dry Matter ppm (mg/kg)	Aqueous extract 1:10 ppm (mg/L)	Dry Matter ppm (mg/kg)	Aqueous extract 1:10 ppm (mg/L)
Na <sup>+</sup>	1020	107.4	628	84.6
ĸ	1398	99.9	1106	74.2
Ca <sup>2+</sup>	246	2.64	315	4.24
Mg <sup>2+</sup>	120	3.075	136	4.08
Zn <sup>2+</sup>	26.4	0.93	33.15	1.34
Mn <sup>2+</sup>	259.5	1.4325	240.4	1.212
Fe <sup>2+</sup>	9.225	0.12	8.87	0.088
A1 3+	6.975	0.4725	8.86	0.62
Cu <sup>2+</sup>	5.475	0.27	8.64	0.35
Pb <sup>2+</sup>	0.0225	lack	0.0314	lack

Table 2 ATOMIC ABSORPTION SPECTROSCOPY FOR GREEN TEA BASE LINE AND BLACK TEA BASE LINE

nicotinamide adenine nucleotides - for the 2 variants sweetened with saccharin (experimental variants V<sub>2</sub> and  $V_{3a}$ ). In these cases, the active centre of saccharin (3<sup>2</sup>-oxo-2,3 dihidro-benzo(d)izotiazol- 1,1-dioxid or 2H-1λ6,2-Benzothiazol-1,1,3-trione - according to the I.U.P.A.C.) oxidizes very much in an anaerobic environment - for both types of teas. A strong oxidizing can be also recorded when using Sucrazit as a sweetener (experimental variants  $V_7$  and  $V_{7a}$ ). In these cases, the saccharine from the sweetener Sucrazit is buffered with sodium bicarbonate - which leads to a milder oxidizing of the environment. The fact that the final product, black tea, passes through the several processes like maturation, fermentation and oxidation (at the conversion from green tea) may also be observed in the unsweetened variants (without additives) and in those where white sugar is added ( $V_{1a}$  and  $V_{2a}$ ) - which have much higher values of NAD concentrations of oxidized forms than the homologous variants of green tea (experimental variants  $V_1$  and  $V_2$ ).

Both aspartame (alone or in synergy with phenyl-alanine from Equal sweetener), and natrium cyclamate (from Edulciclam) oxidize less the black tea environment than the green tea environment - blocking the action of some oxidizing agents existing in this tea (black) even from the fermentation-maturation process (pairs of variants  $V_4$  and  $V_{4a}$ , respectively, pairs of variants  $V_5$  and  $V_{5a}$ ) – figure NAD. The active compounds from honey can change this in oxidation effects; make possible the action block of some oxidants from black tea (see pairs of variants  $V_8$  and  $V_{8a}$ from fig. 2).

The same protective effects in other oxidation processes - in the case of black tea - are recorded at the of black tea

liquid surface (where a series of FAD and FMN dependent enzymes mainly act). In this area, the concentrations of FMN oxidized forms are elevated to the variants that use honey, saccharine, Sucrazit or aspartame as sweeteners (the recorded concentrations were -in descending orderat  $V_{8a}$ ,  $V_{3a}$ ,  $V_{7a}$ ,  $V_{4a}$  – see graphic from figure 3).

On the liquid surface similar concentrations of reduced forms were recorded in both variants sweetened with aspartame, as well as for those sweetened with Zuckli and Sucrazit - in both types of tea, which means that the transformation effects that occur when obtaining black tea from green tea (measured by the concentration of newly formed or modified products) are not influenced by buffered saccharin or aspartame (and this is demonstrated in the comparative analysis of variations in pairs  $V_4/V_{4a} V_6/V_{6a}$ ,  $V_7/V_{7a}$ ).

 $V_{6a}$ ,  $V_7/V_{7a}$ ). Due to these changes (when green tea is transformed in black tea and then sweetened), and, for a better interpretation of the achieved results, the importance of concentrations of oxidized and reduced forms analysis has increased for both NAD and FMN cases.

Analysing the report of concentrations of NAD oxidized and reduced forms, we can see that variants sweetened with saccharin have the highest reports for green tea- in fully active form ( $V_3$ ) and also in the buffered form ( $V_7$ ). In the case of black tea, these reports are much smaller, the forms that are oxidized predominantly in green tea by saccharine are already oxidized (table 3).

The oxidizing potential of the raw black tea is required to be checked in the anaerobic oxidoreductases action area and it is at least two times higher in the variants made with black tea ( $V_{1a}$  and  $V_{2a}$ ) compared with green tea ( $V_{1}$ and  $V_{2}$ ). If white sugar sweetening does not influences significantly the ratio of anaerobic oxidoreductases



Fig.2. The NAD molecular absorption of experimental variants



Table 3				
THE REPORT	[NAD/NAD+H <sup>+</sup> ]	OF EXPERIMENTAL VARIANTS		

Experimental Variants from Green Tea	Report [NAD/NAD+H <sup>+</sup> ]	Experimental Variants from Black Tea	Report [NAD/NADH+H <sup>+</sup> ]
V1 GT Reference (Mt)	0.003	V1a BT Reference (Mt)	0.039
V2 GT+sugar	0.003	V2a BT+sugar	0.055
V3 GT + saccharine	0.067	V3a BT+saccharine	0.003
V4 GT+Aspartame	0.010	V4a BT+Aspartame	0.030
V5 GT+Edulciclam	0.006	V5a BT+Edulciclam	0.010
V6 GT +Zuckli	0.019	V6a BT +Zuckli	0.002
V7 GT+Sucrazit	0.022	V7a BT +Sucrazit	0.019
V8 GT + Honey	0.007	V8a BT+Honey	0.004

Table 4 THE REPORT [FMN/FMNH+H+] OF EXPERIMENTAL VARIANTS

Experimental Variants from Green Tea	Report [FMN/FMNH+H <sup>+</sup> ]	Experimental Variants from Black Tea	Report [FMN/FMNH+H <sup>+</sup> ]
V1 GT Reference (Mt)	3.026	V1a BT Reference (Mt)	8.482
V2 GT+sugar	2.822	V2a BT+sugar	6.635
V3 GT + saccharine	3.322	V3a BT+saccharine	2.364
V4 GT+Aspartame	2.713	V4a BT+Aspartame	4.507
V5 GT+Edulciclam	1.993	V5a BT+Edulciclam	5.360
V6 GT +Zuckli	3.640	V6a BT +Zuckli	5.508
V7 GT+Sucrazit	3.536	V7a BT +Sucrazit	4.249
V8 GT + Honey	1.877	V8a BT+Honey	2.204

coenzymes concentrations - for green tea (experimental variant  $V_2$  compared to experimental variant  $V_1$ ), for the of black tea, the increases of this ratio is by 1.41 times higher (experimental variant V<sub>2</sub> compared to experimental variant  $V_{12}$  (see table 4).

## **Conclusions**

It is very important to study the biochemical and electrochemical processes (practically to study the redox processes catalysed by oxidoreductases in green and black tea) in order to establish the best food additives - according to the best risk management concerning the consumer's health and to improve the consumer's trust for final products alimentary safety.

The ecological agricultural production represents the best basis for the obtaining of functional and replacing foods; in ecological tea, no heavy metals, pesticides, or vegetable growing hormones can be find and this helps to develop a predictive evolution for redox processes and the study of some coenzymes specific for certain oxidoreductases in green and/or black tea. This can represent a main factor to determine and monitor the risks that can occur during some food additive adding processes.

Oxidized forms concentrations of anaerobic oxidoreductases coenzymes are significantly higher for black tea (compared to similar ones in green tea) because of the maturation, fermentation and oxidising processes that the green tea passes in his transformation to black tea

The oxidised potential for raw black tea is necessary to be verified in the action area of anaerobic oxidoreductases and it is at least twice higher for realised variants of black tea  $(V_{1a} \text{ and } V_{2a})$  compared to the one of green tea  $(V_1 \text{ and } V_2)$ V<sub>2</sub>)

Black tea has a smaller antioxidant potential compared to the similar one in green tea (both in raw variants and also in the ones where several sweetener types were used on pair of variants) and this potential difference is generated even from the fermentation, drying, sweeping of green tea (in order to transform it into black tea). These processes come with electron loss and these oxidations reduce very much the concentrations of oxidoreductases reduced forms existing in the tea.

Due to the produced/occurred effects, it can be stated that for green tea sweetening, the best additives are made up with white sugar (as natural sweetener) and Zuckli (as synthetic sweetener). For Zuckli sweetening with (experimental variations  $V_6$  and  $V_{6a}$ ) the sweetening effect is generated by sodium cyclamate - a very good alternative to synthetic sweetener (low in calories).

In order to improve the sensory properties of black tea, the best sweetener was Zuckli. It is very important that we could define (after physical-chemical laboratory research and interpretation of obtained results) the best three variants of sweeteners in this case (black tea): variants sweetened with Zuckli  $(V_{6a})$ , Sugar  $(V_{2a})$  and Edulciclam  $(V_{5a})$ . These variants induce the smallest changes in the black tea basic chemical composition, after the sweetening operation.

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