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STUDY ON THE IMPORTANCE OF REDOX PROCESSES CATALYZED BY NAD- AND FMN- DEPENDENT OXIDOREDUCTASES IN OBTAINING A FOOD SUPPLEMENT BASED ON GREEN TEA

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Abstract
Worldwide, tea is the second most consumed beverage after water. Tea contains active substances that act on the central nervous system, the circulatory system and is a good diuretic. Tea is rich in polyphenols - antioxidants that protect the cells of the human body. In these types of foods, the activity of enzymes such as oxidoreductases, which have NAD- or FMN-dependent coenzymes is very important in this biodynamics.
Tea is usually consumed with sweeteners, both natural and synthetic. These are added to improve sensory qualities. Green tea has a high concentration of chlorophyll pigments, which is maintained at high levels even after boiling. However, this concentration is quite strongly affected by oxidation when some sweeteners were added.
In order to determine the influence of sweeteners on the basic chemical composition, UV-VIS optical spectrometry and mathematical statistics were used.
Key words: NAD, FMN, food supplements, green tea

INTRODUCTION

Green Tea is a "living food", with an extremely valuable chemical composition, in a continuous biodynamics, with the ability to change concentrations in nanoseconds. In these types of foods, the activity of enzymes such as oxidoreductases, which have NAD- or FMN-dependent coenzymes is very important in this bio dynamics [1, 3; 8-10].
The ratios of the concentrations of oxidized and reduced forms of these coenzymes are very important (NAD/NADH $+H^+$ and FMN/FMNH $+H^+$ ratios) in determining the redox potential according to Nernst's equation for each living cell.
Nicotinamide Adenine Dinucleotide (NAD) is a widespread coenzyme in all living cells. One such nucleotide contains an Adenine base and a Nicotinamide base. NAD is present in living cells in two forms: one oxidized and one reduced, abbreviated as NAD^+ and $NADH+H^+$ (H stands for Hydrogen).
Flavin Mononucleotide (FMN) or *Riboflavin-5-Phosphate* is a bio-molecule produced from Riboflavin (vitamin B_2) by the enzyme *Riboflavin-Kinase* and functions as a prosthetic group for several types of oxidoreductases. These types of oxidoreductases also include NADH dehydrogenase, which acts as a cofactor in blue light-sensitive biological receptors [6-8]. During the catalytic cycle there is a reversible conversion of the semiquinone (Semiquinone and FMNH *) and reduced ($FMNH_2$) oxidized forms (FMN) as they occur in several types of oxidoreductases. FMN is a stronger oxidizing agent than

NAD and helps in many processes in which 1 and / or 2 electron transfers occur [11].

NAD Mechanism

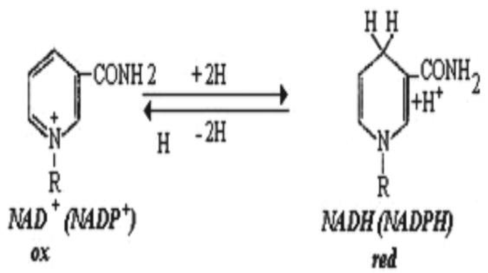

Figure 1. NAD Mechanism of redox processes (Nicotinamide Adenine Dinucleotide) – according Savescu P. 2017 [4,5]

FAD/FMN Mechanism

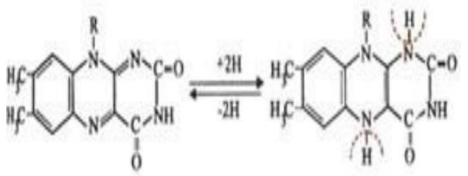

Figure 2. FMN Mechanism of redox processes (Flavin Mononucleotide) – according Savescu P. 2017 [4,5]

MATERIALS AND METHODS

A range of natural and synthetic sweeteners were used in the laboratory analyses to study the effects of these sweeteners on the chemical composition of green tea [6-8].

To obtain the control version of unsweetened green tea, approximately 10 grams of green tea plant per 1000 ml of water was placed in a bowl. The tea was heated, cooled and filtered to produce the experimental V0. This experimental variant was used to create the ten sweetened tea variants. This resulted in the following variants:

V0- unsweetened green tea variety

V1- green tea version sweetened with white sugar

V2- green tea variant sweetened with brown sugar

V3- green tea variant sweetened with honey

V4- green tea version sweetened with saccharin

V5- green tea version sweetened with sucrose

V6- green tea variant sweetened with Diamond

V7- green tea variant sweetened with fructose

V8- green tea variant sweetened with xylitol

V9- green tea variant sweetened with sorbitol

V10- green tea variant sweetened with stevia

Molecular absorption spectra of the proposed experimental variants were obtained using a T92 Plus UV-VIS spectrophotometer manufactured by PG Instruments U.K.

The spectrophotometer was set up to operate at a wavelength bandwidth of 1cm and to record molecular absorption values from nanometre to nanometre in both the UV (190-400nm) and visible (400-700nm) ranges.

The equipment automatically records spectral curves, changing the deuterium and tungsten lamps at 361 nm by automatic programming. To double-check the values obtained, at each measurement the T92 Plus spectrophotometer was set to develop an automatic retracking.

Special parallelepipedal UV quartz cuvettes with a square side in section of 1cm were used to measure absorption.

RESULTS AND DISCUSSIONS

Green tea has a high concentration of chlorophyll pigments, which is maintained at high levels even after boiling. However, this concentration is quite strongly affected by oxidation when brown sugar (experimental variant V2) and saccharin (experimental variant V4) are used as sweeteners.

The use of white sugar in the experimental V1 version produces much smaller changes in the concentration of reduced forms, induces a state of reduced oxidability and is more suitable for consumption by healthy people.

When honey is used as a sweetener, the green tea environment loses transparency and clarity, both color intensity and color tone are affected and a slight oxidizing effect occurs. The influence of honey on the characteristic pigments and theophylline-type alkaloids in green tea varies according to the origin of the green tea and the temperature at which the sweetener is added.

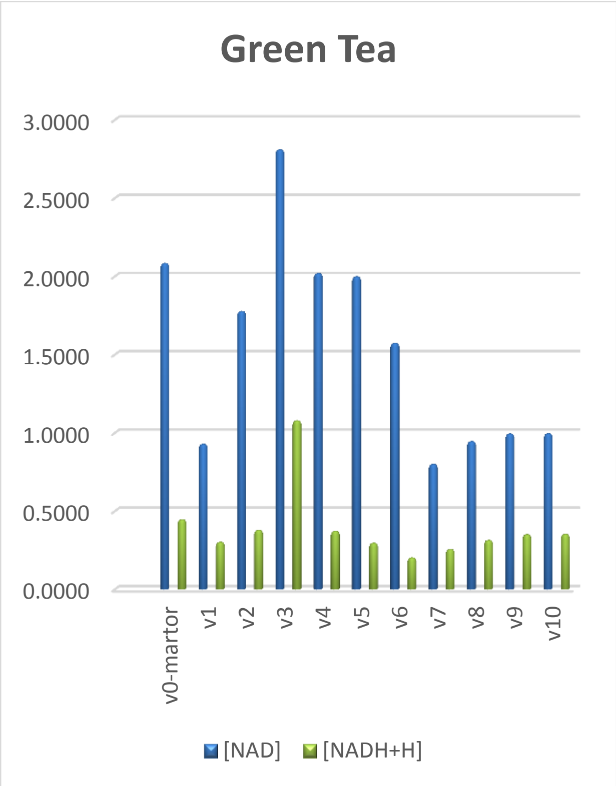

Figure 3. The NAD and NADH+H concentration for experimental variants

The highest concentrations of NAD and NADH+H were obtained in variants V3 (honey) and V4 (saccharin).

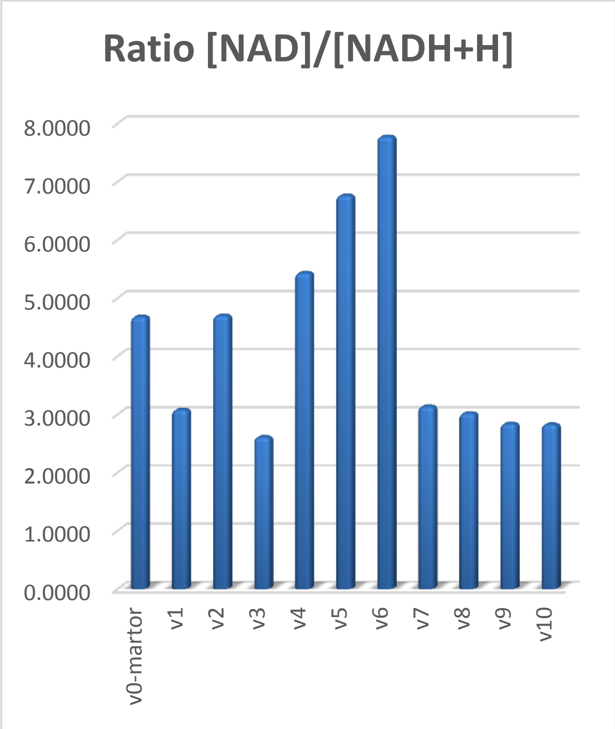

Figure 4. Ratio of concentration for experimental variants

It can be seen that the NAD NADH2 ratio is the highest in the V6 version sweetened with Diamond (sodium saccharin, sodium cyclamate). The use of this sweetener increases the ratio of oxidized and reduced forms of NAD, influencing the redox potential inside the liquid.

Figure 5. Experimental variants

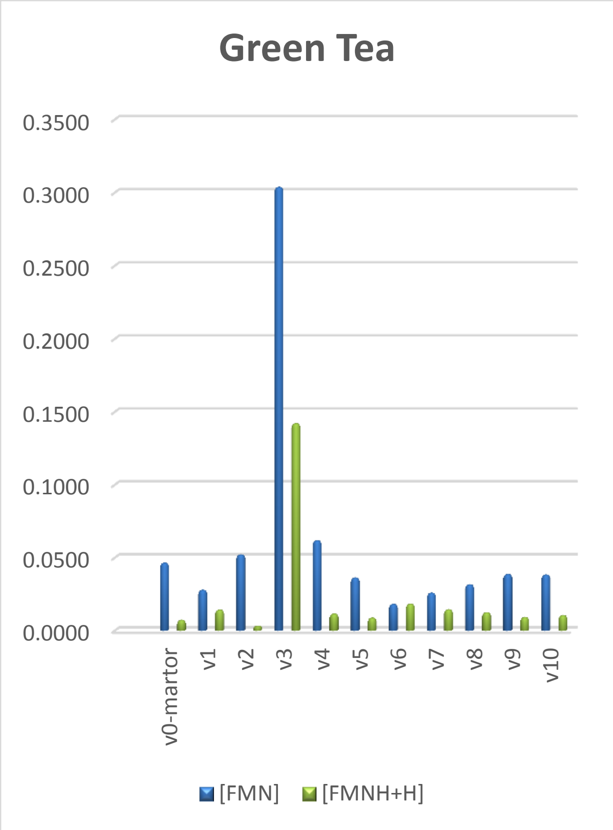

Figure 6. The FMN and FMNH+H concentration for experimental variants

The same increase in concentration is recorded for the honey-sweetened variant (V3) for the concentrations recorded in Figure 6. Honey can create the strongest oxidation on the surface of the sweetened tea, changing also the redox potential value in these variants.

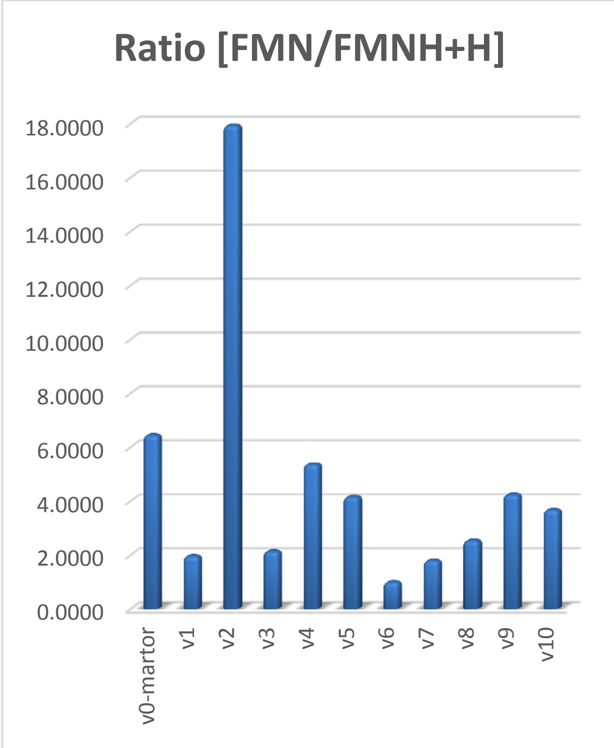

Figure 7. Ratio of concentration for experimental variants

As shown in Figure 7, large differences in the concentration ratios of the oxidised and reduced forms in FMN are found in V2 (brown sugar) and V4 (saccharin).

Crt.Nmb.	Experimental Variant	Coefficient of determination R^2 UV/Visible	Differences (±) from from Mt
1	V1	0,9291/0,911	+0,0552/+0,0567
2	V2	0,9748/0,967	+0,0095/+0,0007
3	V3	0,9993/0,994	-0,015/-0,0263
4	V4	0,9841/0,979	+0,0002/-0,0113
5	V5	0,9788/0,903	+0,0055/+0,0647
6	V6	0,9471/0,7428	+0,0372/+0,2249
7	V7	0,9386/0,8555	+0,0457/+0,1122
8	V8	0,9356/0,916	+0,0487/+0,0517
9	V9	0,962/0,9363	+0,0223/+0,0314
10	V10	0,9626/0,9392	+0,0217/+0,0285
11	V0 – Mt	0,9843/0,9677	0

Tabel 1. The R^2 statistical coefficient of experimental variants. The registered differences vs. witness variant

CONCLUSIONS

A number of clear conclusions emerge from analysing the laboratory results and interpreting the values:

Green tea has a high concentration of chlorophyll pigments, which is maintained at high levels even after boiling. However, this concentration is quite strongly affected by oxidation when brown sugar is used as sweetener (experimental variant V2) and saccharin (experimental variant V4).

The use of white sugar in the experimental V1 version produces much smaller changes in the concentration of the reduced forms, induces a state of reduced oxidability, and is more suitable for consumption by healthy people.

The use of sucralfate in the experimental version of V5 gives a significantly improved result, which is beneficial for people with digestive, diabetic or cardiovascular problems and is recommended for these categories of consumers.

For healthy consumers, the best variant is the one with white sugar, experimental variant V1 (also proven by the difference of the R2 coefficients of determination with the control), and for consumers with health problems, the best variant is experimental variant V5, in which the sweetener sucrose was used.

ACKNOWLEDGEMENTS

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