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

Author(s) Constantin BUTOI[1], Alexandra Ioana IONESCU[1], Viorel SCHIOPU[1], Georgeta POPESCU[1]

Institutions *(1)University of Life Sciences "King Mihai I" Timișoara, IRVA Doctoral School, 119 Calea Aradului, Timișoara, Romania*

author email: butoiconstantin@yahoo.com

Corresponding author email: ialexandra_08@yahoo.com

Abstract

Tea consumption worldwide is on the rise and is one of the most popular beverages worldwide. Black tea is a stimulant because it contains active substances that act on the central nervous system, the circulatory system and has a diuretic action. The antioxidants found in this type of tea neutralize oxygen free radicals, which attack the body's cells and degrade their functioning. Black 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- (Nicotinamide adenine dinucleotide) or FMN- (Flavin mononucleotide) dependent coenzymes is very important in this biodynamics. To enhance the sensory qualities of black tea, the main food additives used are natural and synthetic sweeteners. 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 work at a wavelength bandwidth of 1cm and record molecular absorption values from nanometre to nanometre in both the UV range (190-400 nm) and the visible range (400-700 nm).

Key words: NAD, FMN, food supplements, black tea

INTRODUCTION

Black 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 B2) 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. During the catalytic cycle there is a reversible conversion of the semiquinone (Semiquinone and FMNH *) and reduced (FMNH2) 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].

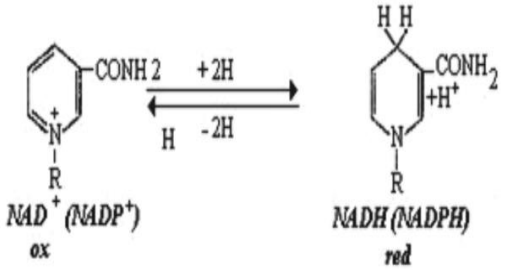

Figure 1. The NAD mechanism of redox processes (NAD- Nicotinamide Adenine Dinucleotide)- according Savescu P, 2017 [4,5]

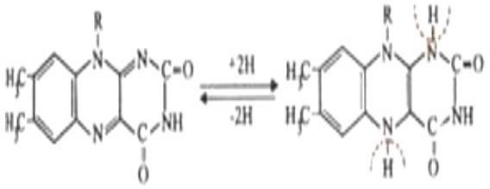

Figure 2. The FMN mechanism of redox processes (FMN- Flavine Mononucleotide)- according Savescu P, 2017 [4,5]

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 black tea [6-8]..

In order to obtain the control version of the unsweetened black tea, approximately 10 grams of the black tea plant per 1000 ml of water were placed in a bowl. The tea was then heated, cooled and filtered to produce the experimental variant V0. From this experimental variant, the ten sweetened tea variants were made. This resulted in the following variants:

V0- unsweetened black tea variety
V1- black tea version sweetened with white sugar
V2- black tea version sweetened with brown sugar
V3- black tea variant sweetened with honey
V4- black tea version sweetened with saccharin
V5- black tea version sweetened with sucrose
V6- black tea version sweetened with Diamond (sodium cyclamate and sodium saccharin)
V7- black tea variant sweetened with fructose
V8- black tea variant sweetened with xylitol
V9- black tea version sweetened with sorbitol
V10- black tea version sweetened with stevia

To facilitate the passage of the incident light beam through the tea solution, a dilution of 1:50 (2%) was used. This dilution was obtained by sieving after filtering the black tea and passing it through the cuvettes of the spectrophotometer.

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 work at a wavelength bandwidth of 1cm and record molecular absorption values from nanometre to nanometre in both the UV range (190-400 nm) and the visible range (400-700 nm).

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

RESULTS AND DISCUSSIONS

For the V0 variant we have a peak of NAD (Nicotinamide adenine dinucleotide) a decrease in tocopherol content and a high theobromine content.
The white sugar introduced in V1 induces small changes in the content of pigments and a slight decrease in the content of certain alkaloids, in particular theobromine. The tendency to modify the flavoproteins is also observed when using brown sugar in V2, but more on the oxidised forms and in this case the influence on the pigments is much lighter. So brown sugar even though it alters the colour intensity and colour tone protects the reduced forms of flavoprotein, it is a better variant than white sugar sweetening.
For healthy consumers the best sweetening variant is the one using white sugar V1 (proven by the difference of the R^2 coefficients of determination from the control) and for consumers with health problems the best variant is V8 sweetened with xylitol.

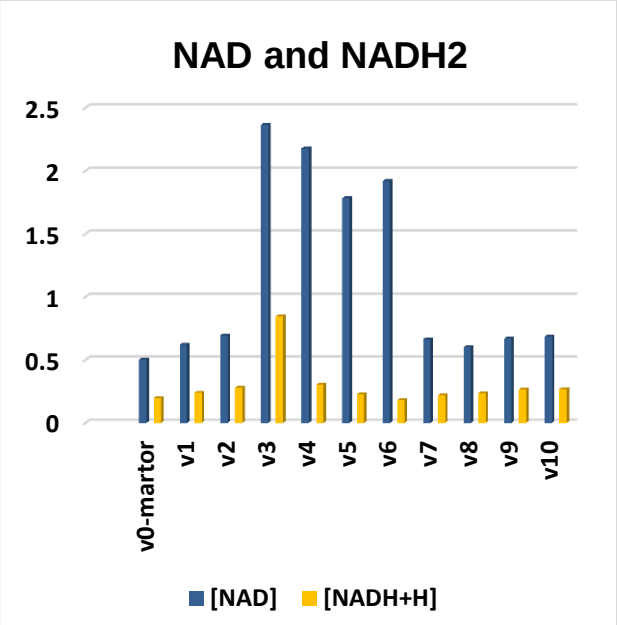

Figure 3. The NAD and NADH2 concentration for experimental variants

The highest concentrations of NAD and NADH2 were obtained in v3 (honey) and v4 (saccharin)

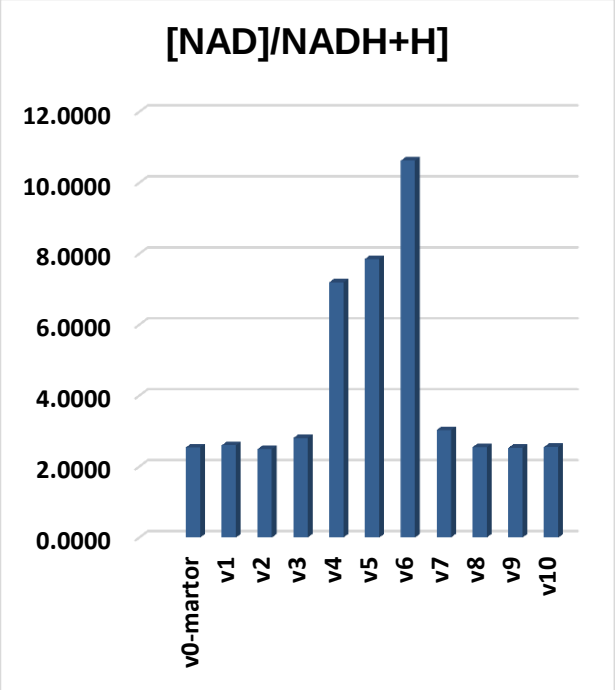

Figure 4. Ratio of concentration for experimental variants

It can be seen that the NAD/NADH2 ratio is the highest in the v6 version sweetened with Diamond (sodium cyclamate and sodium saccharin). The use of this sweetener increases the ratio of oxidized and reduced forms of NAD, influencing the redox potential inside the liquid (sweetened black tea).

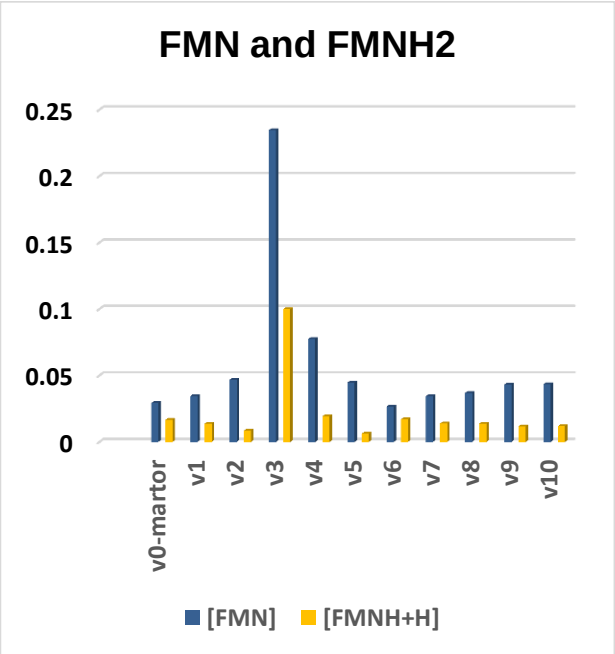

Figure 5. The FMN and FMNH2 concentration for experimental variants

The same increase in concentration is recorded for the honey-sweetened variant (v3) for the concentrations recorded in Figure 5. Thus honey can create the strongest oxidation on the surface of the sweetened tea, changing also the redox potential value in these variants.

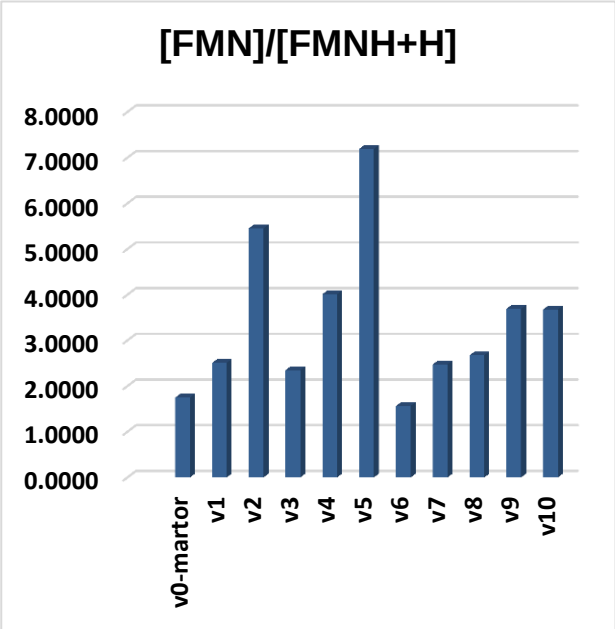

Figure 6. Ratio of concentration for experimental variants

As shown in Figure 6, large differences in the concentration ratios of the oxidised and reduced forms in FMN are found in V2 (brown sugar) and V5 (sucrose).

Figure7. Experimental variants

Nr. crt.	Variante experimentale	Coeficientul de determinare R^2 UV/Vizibil	Diferențele (±) înregistrate față De valoarea Mt
1	V1	0,9561/0,9797	+0,0067/-0,0057
2	V2	0,9697/0,9846	-0,0069/-0,0106
3	V3	0,9737/0,9953	-0,0109/-0,0213
4	V4	0,9066/0,9845	+0,0562/-0,0105
5	V5	0,9031/0,978	+0,0597/-0,004
6	V6	0,9568/0,9716	+0,006/+0,0024
7	V7	0,9615/0,9778	+0,0013/-0,0038
8	V8	0,9616/0,98	+0,0012/-0,006
9	V9	0,9609/0,9832	+0,0019/-0,0092
10	V10	0,9603/0,9834	+0,0025/-0,0094
11	V0 - Mt	0,9628/0,974	0

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

CONCLUSIONS

By analysing the results obtained and interpreting the values provided, a number of important conclusions can easily be drawn:

Brown sugar, even though it alters colour intensity and hue, protects the reduced forms of flavo-protein which makes it a better option than white sugar.

Honey used as a sweetener in black tea induces a higher oxidation state and changes the clarity of the tea.

Sucrose has a strong effect on the concentration of the main active compounds found in black tea.

Sucrazite becomes more readily usable by consumers by buffering the active saccharin core, inducing the sweet taste under conditions of reduced oxidability.

The use of sodium cyclamate and saccharin mixture ensures a certain degree of preservability of the concentration of the hydrogenated forms of specific oxidoreductase coenzymes while preserving their strong antioxidant character.

ACKNOWLEDGEMENTS

-

REFERENCES

Savescu, P. et all. (2006). Environmental risk's decreasing according to the European Standard (QMS ISO 9000:2000) applied to food factories, *JEPE* , 2006, *Vol 6 (2),* 447-453.

Savescu, P. (2016). Improving the concentrations of bioactive compounds (with antioxidant properties) in alfalfa and corn for their use in food supplements and functional food. *Nano, Bio and Green-Technologies for a Sustainable Future, SGEM International Conference, Albena, BG, No.6, (1),* 583-590.

Savescu P., Poenaru, M.M, Iacobescu F. (2016). Study regarding the Development of Organic Farming Systems in Romania as the Basis for obtaining of innocuity Agricultural Raw Materials – used in Functional Food, *Annals of the University of Craiova, Series Agriculture, Montanology, Cadastre, No.1, 46,* 281-285.

Savescu P. (2017). A New Approach to Study the Sweetener's Effect on Green Tea Oxidative Status, *Revista de Chimie,* 68 (2), 294-299.

Savescu, P. **(**2017), Comparative Study on the Effect of Sweeteners on the Oxidative Status of Green Tea and Black Tea, *Revista de Chimie, 68 (6),* 1406-1412.

Savescu, P., (2017). A New Approach to Study the Sweetener's Effect On Hibiscus Tea Oxidative Status, *International Conference SGEM 2017, Section 25 - Advances in Biotechnology, Albena, Bulgaria,* DOI: 10.5593/sgem2017/61/S25.059, ISSN 1314-2704, Vol 17, Nr. 1, Rev. 61, 455-460.

Savescu, P., Badescu, G., Milut, M., Ciobanu, A., Apostol, L., Vladut, V. (2019).Healthy Food – Through Innovative Technologies*. Vol. ISB-INMA-TEH Bucharest.* 516-521.

Savescu, P., Iacobescu, F., Poenaru, M.M. (2020). Study on the Use of Biomaterials as Protective Membranes for Certain Functional Foods*.* In Book *Advances in Bionanomaterials II*, *Book Series: Lecture Notes in Bioengineering. Publisher: Springer International Publishing, Print ISBN: 978-3-030-47704-2, Springer Nature Switzerland AG 2020, S. Piotto et al. (Eds.),BIONAM 2019,LNBE,* https://doi.org/10.1007/978-3-030-47705-9_16, 1-13.

Savescu, P., Iacobescu, F., Poenaru, M.M. (2020). *Patent Application -OSIM A00745, ” Innovative food supplement based on plant products obtained from organic farming”*, OSIM, Romania.

Savescu, P. (2021). Chapter Natural Compounds with Antioxidant Activity-Used in the Design of Functional Foods. In *Book Title -Functional Foods - Phytochemicals and Health Promoting Potential,* IntechOpen Eds., London,U.K., *DOI:* http://dx.doi.org/10.5772/intechopen.97364, 1-25.

Tita, O., E Lengyel., E., Stegarus, D., Savescu, P., Ciubara, A.B., (2021). Identification and Quantification of Valuable Compounds in Red Grape Seeds, *Appl. Sci. 11*(11), 5124; https://doi.org/10.3390/app11115124, 5124-5139.