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VARIATION OF FLAVOPROTEIN CONCENTRATION AFTER SWEETENING IN A GREEN-TEA BASED FOOD SUPPLEMENT

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Abstract

Flavoproteins are proteins that contain a nucleic acid derivative of riboflavin. Flavoproteins removes the free radicals that contributes to oxidative stress and have an important role in DNA repair. The raw material used, is free from plant growth hormones and pesticides, helping to maintain high levels of nutrients in the finished product. The objective of this paper work is to identify the variant with the highest concentration of flavoproteins following the green tea sweetening process, in order to produce the most effective product for an innovative food supplement. The results of this phase of research have identified the variant with the highest concentration of flavoproteins following the green tea sweetening process. The best variant obtained at this stage of research is characterized by sensory improvements and minimal changes in chemical composition compared to the unsweetened variant.

***Key words**: enzyme, flavoprotein, green tea, sweeteners.*

INTRODUCTION

Tea is one of the most consumed beverages. Beneficial effects on the circulatory system, improvement of heart function, prevention of atherosclerosis and obesity, anticancer, anti-inflammatory, and antibacterial properties are some of the properties of tea. Most of them result from the presence of biologically active substances in tea leaves, mainly polyphenolic compounds, but also alkaloids (theobromine, theophylline, and caffeine) and theanine (Savescu, 2016a; Savescu, 2021; Kowalska et al., 2021).

Abbas & Sibirny write in their paper that riboflavin [7,8-dimethyl-10-(1′-d-ribityl) isoalloxazine, vitamin B_2] is an obligatory component of human and animal diets, as it serves as a precursor of the flavin coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are involved in oxidative metabolism and other processes (Abbas & Sibirny, 2011; Tița et al., 2021).

Succinate dehydrogenase is an example of a flavoenzyme; it catalyzes the removal of two hydrogen atoms from the succinic acid molecule and their transfer to the prosthetic FAD group. Many of these flavoenzymes additionally contain a metal, which is essential for the function of metal-flavoprotein enzymes (Savescu et al., 2019; Savescu et al., 2020b).

Nobel laureate Szent-Györgyi showed that flavoproteins act as electron carriers between dehydrogenases and iron-containing cytochromes. The segments of the electron transport chain have been reconstituted in vitro using purified components (Isenberg & Szent-Györgyi, 1958).

NAD-dependent oxidoreductases are enzymes from the class of anaerobic dehydrogenases and have as coenzymes, Nicotinamide Adenine Dinucleotide (NAD^+) or reduced ($NADH+H^+$) and Nicotinamide Adenine Dinucleotide Phosphate Oxidate ($NADP^+$) or reduced ($NADPH+H^+$). These coenzymes consist of a derivative of vitamin PP, nicotinamide and an adenine-derived nucleus (Savescu, 2021).

NAD^+ and $NADP^+$ are anaerobic, because the transferred hydrogen acceptor is not oxygen, but another element. They catalyze redox reactions by the generally reversible transfer of protons. The transfer of hydrogen in the redox reactions catalyzed by NAD^+ and $NADP^+$ is carried out at the level of the nicotinamide component in the structure of these coenzymes (Savescu, 2021).

In certain liquid foods, these mechanisms can be disturbed by certain food additives (sweeteners, preservatives, colorings). Sweeteners can have an oxidative effect on these redox systems, and this effect can significantly reduce the antioxidant capacity of the food. In green tea, sweeteners can reduce the concentration of reduced active forms of some oxidoreductases (in particular metal-flavoprotein enzymes) (Savescu, 2019; Savescu, 2021).

$NADH+H^+$ is the form in which electrons are collected from various substrates by NAD^+-dependent dehydrogenases. These electrons are passed up the respiratory chain via flavoproteins (NADH dehydrogenases) (Savescu, 2021).

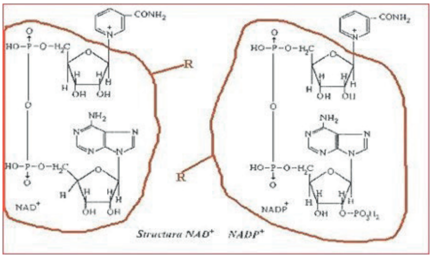

Figure 1. Structure of NAD^+ and $NADP^+$ - according Savescu, 2021

There are two types of flavoproteins with dehydrogenase activity. L-amino acid oxidase is localized in the endoplasmic reticulum and D-amino acid oxidase is localized in the microsomes of liver cells (Savescu, 2016b; Savescu, 2017b).

The flavin nucleotides of these oxidases ($FADH_2$ and $FMNH_2$) react directly with molecular oxygen, generating H_2O_2 which is in turn broken down into water and oxygen under the influence of catalase. This enzymatic process occurs in the peroxisomes of liver cells. FMN (Flavin mononucleotide) is a prosthetic (protease) group of flavoprotein. It is similar in structure to FAD (Flavin adenine dinucleotide), but lacks adenine as a nucleotide. FMN (like FAD) can accept 2 e- + 2 H+ to yield $FMNH_2$. When bound to the active site of some enzymes, FMN can accept e-, converting to a semiquinone radical (Savescu, 2017a).

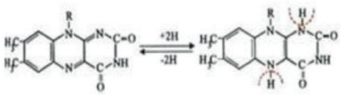

Figure 2. FMN Mechanism of redox processes (Flavin Mononucleotide) (Savescu, 2017)

FAD-dependent oxidoreductases are enzymes of a heteroproteinic nature from the group of aerobic dehydrogenases having as active groups derivatives of vitamin B2 (riboflavin or 7,8-dimethyl-10-ribithyl-isoaloxazine), namely: flavin adenine mononucleotide (FMN) and flavin dinucleotide (FAD) (Savescu, 2021). Flavin enzymes (FMN, FAD) are involved in electron and proton transfer reactions mediated by the isoalloxazine nucleus. They accept either an electron or a pair of electrons (unlike NAD^+ and $NADP^+$ which only accept electron pairs) (Savescu, 2021).

Flavinenzymes have the standard redox potential Eo between +0.19V (oxidants stronger than NAD^+) and –0.49V (reducing agent stronger than NADH), which shows a wide range of variation of redox properties depending on environmental conditions and the nature of the substrate (Savescu, 2021).

Theophylline is a dimethylxanthine having the two methyl groups located at positions 1 and 3. It is structurally similar to caffeine and is found in green and black tea. It has a role as a vasodilator agent, a bronchodilator agent, a muscle relaxant, an EC 3.1.4.* (phosphoric diester hydrolase) inhibitor, an anti-asthmatic drug, an anti-inflammatory agent, an immunomodulator, an adenosine receptor antagonist, a drug metabolite, a fungal metabolite and a human blood serum metabolite (National Center for Biotechnology Information, 2025).

The main objective was to determine which of the sweeteners could induce changes in the concentrations of the active forms of flavoproteins in green tea.

Using a statistical program such as origin pro 2020, positive correlations were found between the concentrations of reduced forms of some oxidoreductases and certain sweeteners (brown sugar) and negative correlations (sucrose, fructose) between other sweeteners and these reduced forms. Most previous studies have concerned an analysis of the positive/negative correlations between certain NAD- and NADP-dependent oxidoreductases and certain sweeteners (Savescu, 2020b).

This paper aimed to analyze the variation of flavoprotein concentrations in tea upon additivities with certain natural and synthetic sweeteners using analytical methods. These data will be further statistically analyzed using an updated statistical software.

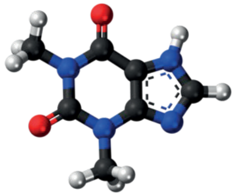

Figure 3. Chemical structure of theophylline
https://en.wikipedia.org/wiki/Theophylline#/media/File:
Theophylline_3D_ball.png

MATERIALS AND METHODS

A range of natural and synthetic sweeteners were used in the laboratory to study the effects of these sweeteners on the chemical composition of green tea. The raw material utilized (green tea) was subjected to testing using A.A.S. (Atomic Absorption Spectroscopy), with the purpose of determining the presence of any contamination by residues or heavy metals. The influence of sweeteners on the flavoprotein activity was ascertained through the implementation of UV-VIS optical spectrometry and mathematical statistics. Approximately 10 grams of green tea plant per 1000 ml of water was added to a bowl to obtain the control version of unsweetened green tea. The tea was heated, cooled and filtered to produce the experimental V0. The present experiment involved the creation of ten sweetened tea variants, which were derived from the experimental variant (Figure 4). The resulting variants are listed below:

V0 - an unsweetened green tea variety;
V1 - green tea variety sweetened with white sugar;
V2 - variant of green tea sweetened with brown sugar;
V3 - green tea variant sweetened with honey;
V4 - green tea variant sweetened with saccharin;
V5 - green tea sweetened with Sucrazit variant;
V6 - variant of green tea sweetened with Diamond;
V7 - variant of green tea sweetened with fructose;
V8 - variant of green tea sweetened with xylitol;
V9 - green tea sweetened with sorbitol;
V10 - green tea sweetened with stevia.

The experimental version V5, Sucrazit, is the trade name of the synthetic sweetener containing 20% saccharin, citric acid, whose acidity has been buffered with sodium bicarbonate.

The experimental variant V6, Diamond, is the trade name for a synthetic sweetener containing a combination of sodium cyclamate and sodium saccharine.

Molecular absorption spectra of flavoproteins from experimental variants were obtained using certified reference substances and a T92 Plus UV-VIS spectrophotometer manufactured by PG Instruments U.K.

Certified reference substances (pure analytical substances) were used to make calibration scales of different concentrations, which helped to find the wavelength at which the maximum molecular absorption spectra for flavoproteins and riboflavin are recorded. Using the single addition method and interpolation on both axes, specific wavelengths were determined.

As we pointed out in a previous paper, the spectrophotometer was configured to function within a wavelength bandwidth of 1 cm, thereby enabling the measurement of nanometre-to-nanometre molecular absorption values across the UV (190-400 nm) and visible (400-700 nm) ranges. This configuration permitted the acquisition of data in both the UV (190-400 nm) and visible (400-700 nm) ranges (Ionescu et al., 2023).

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 (Ionescu et al., 2023).

Absorbance measurements were made in special quartz UV cuvettes.

The first stage of sample preparation for atomic absorption spectrometry is mineralization (microwave digestion) (Savescu, 2017c). Microwave digestion is a method of sample preparation for elemental analysis by ICP, ICP-MS or AA. It is applicable to a wide range of samples, including those from plant, soil, food and pharmaceutical sources. The principle behind this method is to dissolve the sample before introducing it into the analyzer. In contrast, acid digestion is used to break down the sample matrix, leaving the elements of interest in solution and ready for analysis (Savescu, 2017c). CEM microwave digestion systems rapidly break down a wide variety of sample matrices, leaving behind a clear solution containing the analytes of interest. (Savescu, 2017c).

The experiment employed a 1200 W MARS mineralized microwave CEM system, a multimode platform equipped with a magnetic stir plate and a rotor that facilitates parallel processing of multiple vessels per batch. The MARS CEM system is notable for its versatility, offering a range of modes that include a Teflon insert (TFA) (HP-500) (80 ml vessel volume, max pressure 350 psi, max temperature 210°C) and Greenchem (glass insert (borosilicate)) (80 ml vessel volume, max pressure 200 psi, max temperature 200°C) vessel types, both based on a 14-position rotor (Savescu, 2017b). The system provides a constant power output ranging from 0 to 1200 watts. The temperature is regulated internally by a fiber optic probe situated within a control reference vessel (Savescu, 2017b).

Method: In summary, 10 g of tea solids were measured with analytical precision. For the mineralization step, 10.00 g of product (green tea), 6 ml of concentrated nitric acid and 3 ml of 30% hydrogen peroxide were added to each digestion cartridge. A digestion cartridge containing no product but only reagents was used as a control (L'vov, 2005; Belitz, 2009; Savescu, 2017b).

Figure 4. Experimental variants

RESULTS AND DISCUSSIONS

During the stages of obtaining innovative dietary supplements from green tea, it is recommended to monitor the active forms of flavoproteins and riboflavin in green tea additivities to maintain the antioxidant capacity of the final product. When monitoring and controlling subsequent products, only those experimental variants that show the smallest changes in flavoprotein and riboflavin concentrations compared to the control variants after sweetening should be emphasised. These will also be the variants recommended to consumers, especially those with diabetes or nutritional disorders.

The use of the AAS technique revealed high concentrations of potassium, magnesium and calcium in the parts of the tea plant used, thus increasing the nutritional density of the final product - green tea dietary supplement.

Table 1. Atomic Absorption Spectroscopy baseline for green tea

Indicator/ Constituent	GREEN TEA	
	Dry matter ppm (mg/kg)*	Watery extract 1:10 ppm (mg/L) Average value
Na^{+}	872±1.74	77.86
K^{+}	1484±1.86	72.43
Ca^{2+}	376±0.96	2.04
Mg^{2+}	212±0.88	2.10
Zn^{2+}	24.6±0.02	0.62
Mn^{2+}	288±0.94	0.95
Fe^{2+}	8.74±0.03	0.08
Al^{3+}	6.86±0.03	0.31
Cu^{2+}	4.267±0.03	0.18
Pb^{2+}	0.018±0.002	lipsa

V3 (honey) and V4 (saccharin) gave the highest concentrations of NAD^{+} and $NADH+H^{+}$ and FMN and $FMNH_2$.

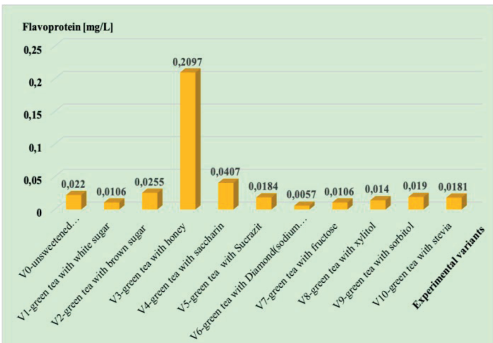

Figure 5. Green tea flavoprotein concentration

The flavoprotein concentrations reached the highest value in the V3 variant (honey) and the lowest one in the V6 variant (sodium cyclamate and saccharin).

Honey (used in the experimental variant V3) exerts a strong effect on flavoproteins in green tea. According to the graphs, increased concentrations of the oxidized and reduced forms of riboflavin in green tea are present in this variant (V3 - Figures 5, 6, 7). It is thus proven to increase the flavoprotein activity of green tea when honey is used as a sweetener.

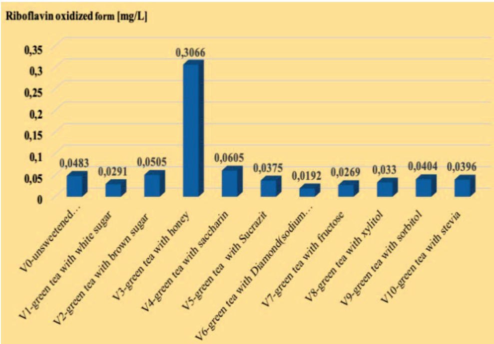

Figure 6. Concentration of the oxidized form of riboflavin in green tea

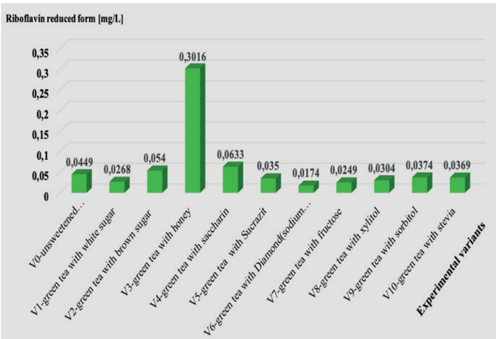

Figure 7. Concentration of the reduced form of riboflavin in green tea

Saccharin induces strong oxidative effects in the chemical composition of green tea and alters both the hue and intensity of the colour, causing visible disturbances. When Sucrazit is added to the medium, the oxidation effects are much less as the coloured pigments are better protected. The additional activation of the isoalloxazine nucleus of riboflavin was proved when saccharin was acidified with an organic acid and buffering of this nucleus with bicarbonate (Sucrazit sweetened variant). Evidence for this phenomenon has been demonstrated in the case of oxidoreductase activity within green tea (Ionescu, 2023).

A decrease in the concentration of active forms of riboflavin can be observed when fructose (V7) is used (Figures 6, 7).

The experimental variants V8 and V9 with sorbitol and xylitol showed similar variations of the molecular absorption spectra curves, inducing almost the same effects as the unsweetened variant (V0).

CONCLUSIONS

From analyzing the results of the laboratory experiments and interpreting the values, a number of clear conclusions emerge.

Brown sugar (V2) produce the smallest alterations in the concentration of reduced forms and induces a reduced oxidizable state.

Honey (V3) denatures the sweetened variant the most compared to the control variant (V0).

The use of honey (V3) and saccharin (V4) increases the concentrations of some NAD- and FMN-dependent coenzymes.

The same trends are seen for flavoprotein concentrations (the highest concentrations were found in the V3 variant - honey and the lowest in the V6 variant – Diamond).

The use of fructose (V7) induces a decrease in the concentrations of the active forms of riboflavin.

Sorbitol (V8) and xylitol (V9) produce minimal changes, very close to the control variant.

Although sweetened products benefit from improved sensory qualities, sweetening must be done carefully so that valuable biocompounds are not altered. Consequently, the optimal sweetener is that which generates minimal alterations in the chemical composition, whilst preserving all the advantageous characteristics of green tea. This sweetener is brown sugar (V2).

It is inadvisable to use sweeteners containing saccharin and sodium cyclamate to sweeten green tea. This is because these sweeteners (Diamond, trade name) have been shown to produce very high changes in the activity of flavoprotein.

The development of innovative methods and technologies for the management of food additives facilitates the production of high-quality and safe food end products.

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