VARIATION OF HEMIN PROTEIN CONCENTRATION IN GREEN TEA AFTER SWEETENING

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Abstract

Green tea is obtained by processing the leaves of the Camellia sinensis plant. Tea contains active substances that act on the central nervous system, the circulatory system and is a good diuretic. It is rich in mineral salts and vitamins, especially vitamin C, and helps to strengthen the immune system. Tea leaves contain tannins, amino acids, carbohydrates and essential oils that give the tea its colour and flavour. Green tea is recognized for its high antioxidant potential, being very rich in polyphenolic compounds, vitamins, and pro-vitamins.

Chlorophyll is remarkably similar in structure to hemoglobin, the oxygen-carrying pigment found in the red blood cells of mammals and other vertebrates

Hemoproteins are the photosynthetic and respiratory cytochromes, involved in electron transfer, and the globin that bind oxygen.

In order to determine the variation of heme protein concentration in green tea after sweetening, UV-VIS optical spectrometry and mathematical statistics were used.

Key words**: **green tea, hemin protein, chlorophyl

INTRODUCTION

People have benefited from drinking green tea for centuries. Some of the most important effects of the consumption of the green tea are stress relief, increase of skin health, it helps with weight loss and also reduce the risk of cardiovascular disease.

Green tea is obtained from the leaves of *Camellia sinensis* plant, that is having it's origins in Asia, being rich in chlorophyll antioxidants and polyphenols due to the lack of leaves oxidation and minimal processing process.

Chlorophyll is a complex green pigment found in plants, algae, and certain bacteria, plays a crucial role in the process of photosynthesis by absorbing light energy and converting it into chemical energy.

Chlorophyll is a complex molecule made up of a porphyrin ring, a magnesium ion, and an attached hydrocarbon tail. The porphyrin ring is responsible for absorbing light energy and the magnesium ion acts as an electron acceptor. Chlorophyll has many forms such as chlorophyll *a*, chlorophyll *b*, chlorophyll *c*, chlorophyll *d* and chlorophyll *e*. Chlorophylls *a* and *b* are the major types found in higher plants and green algae; chlorophylls *c* and *d* are found, often with *a*, in different algae; chlorophyll *e* is a rare type found in some golden algae; and bacterio-chlorophyll occurs in certain bacteria. In green plants chlorophyll occurs in membranous disklike units (thylakoids) in organelles called chloroplasts.

Chlorophyll is remarkably similar in structure to hemoglobin, the oxygen-carrying pigment found in the red blood cells of mammals and other vertebrates.

Hemoglobin is a heme protein, the iron-containing protein in the blood of many animals, in the red blood cells (erythrocytes)

of vertebrates, that transports oxygen to the tissues.

Hemoproteins are the photosynthetic and respiratory cytochromes, involved in electron transfer, and the globin that bind oxygen. Other examples include the oxidative enzymes catalase, peroxidase, and NADPH oxidase, involved in the production and/or scavenging of free radicals, and the very large group of cytochrome P450 enzymes. In plants, hemoproteins are distributed in all subcellular locations, but the situation is made more complex by the fact that heme is synthesized in both mitochondria and chloroplasts, and it is not known which organelle supplies heme to other users in the cell.

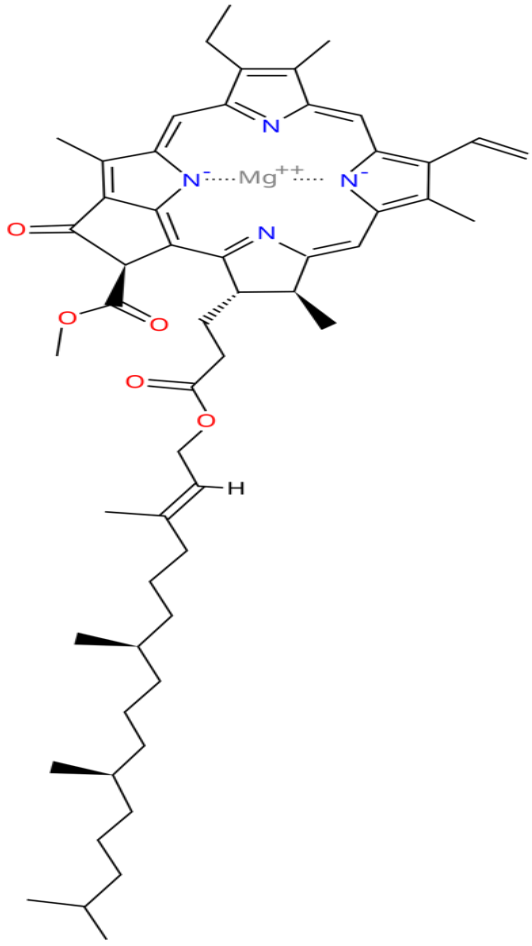

Figure 1. Chlorophyll *a*

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.

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.

The single addition method allowed us to identify the range in which the highest values of specific molecular absorption spectra are obtained for green tea. The proposed method represents a new alternative for measuring the redox status of green tea. Corrections were made for temperature, pH and water activity, resulting in the most representative wavelengths for observing the oxidised and reduced forms of cytochrome P450 oxidase. The concentration of the oxidised and reduced forms directly affects the redox potential value for each experimental variant (according to the Nernst equation).

$$E = E^{o} + (0.059/z)\log\frac{[oxidised\ species]}{[reduced\ species]}$$

RESULTS AND DISCUSSIONS

As it can be seen in the following graphics, the best sweetening option are brown sugar (V2), that can be consumed by healthy people, and sucrose (V5), which can be consumed by people with digestive, diabetic or cardiovascular problems. There is an influence on the oxidised forms and a slight influence on the pigments in the case of brown sugar, but even if it changes the intensity and tone of the colour, it protects the reduced forms.

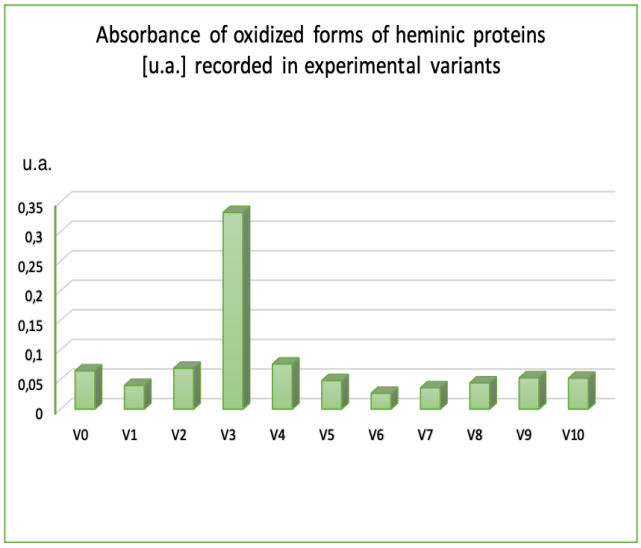

Figure 2. Absorbance of oxidized forms of heminic proteins [u.a.] recorded in experimental variants.

The highest absorbance of oxidized forms of heminic proteins is for experimental variant V3 (honey). 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 thiophilin-type alkaloids in green tea varies according to the origin of the green tea and the temperature at which the sweetener is added.

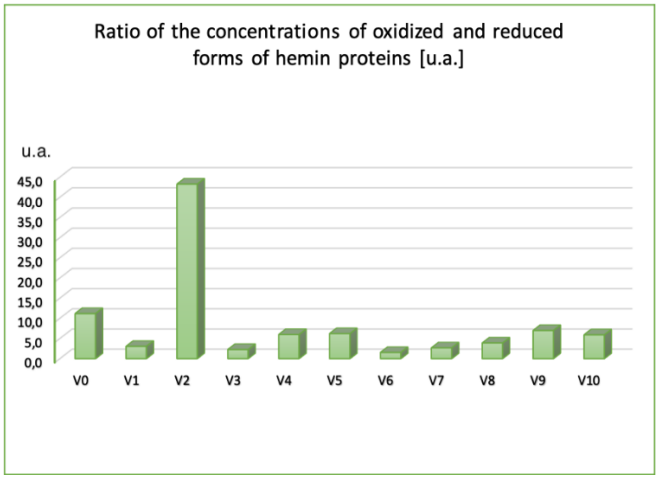

Figure 3. Ratio of the concentration of oxidized and reduced forms of heminic proteins.

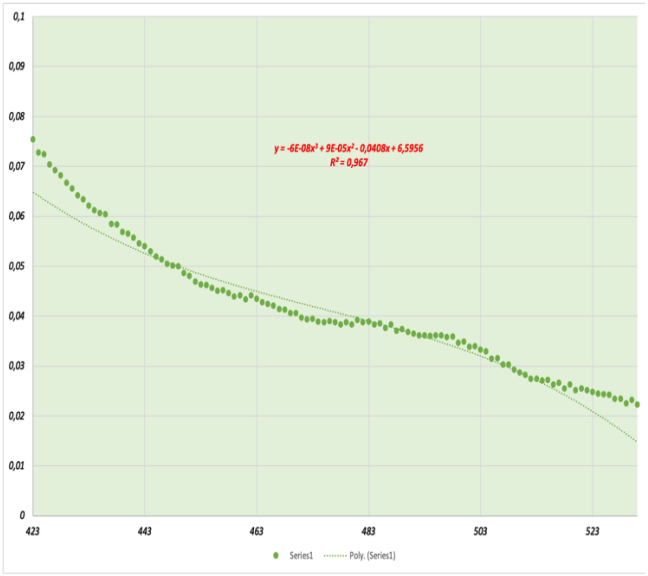

Figure 4. Variation in absorbance (u.a.) for V2 in the visible range

Analysing these graphics it can be seen that the best sweetening variant was brown sugar inducing a higher ratio.

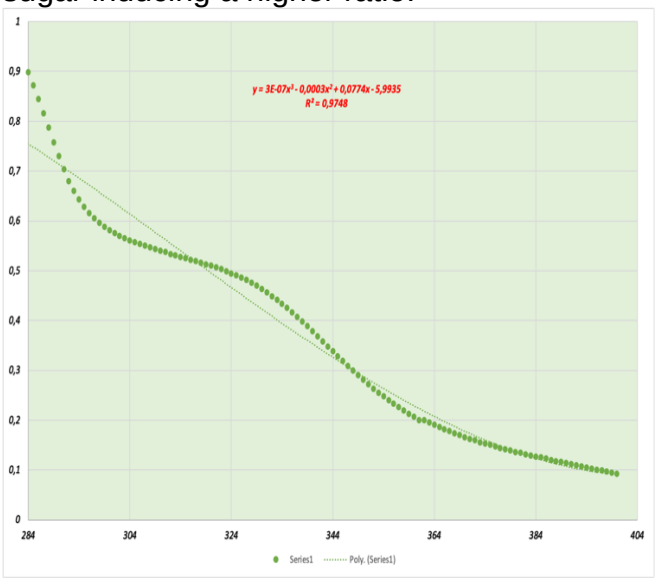

Figure 5. Variation in absorbance (u.a.) for V2 in the UV range

CONCLUSIONS

Analysing the results of the laboratory experiments and the interpretation of the values led to following clear conclusions: Green tea has a high concentration of chlorophyll pigments, which is maintained at a high level even after boiling. However, this concentration is significantly affected by oxidation when white sugar (experimental variant V1) and saccharin (experimental variant V4) are used as sweeteners.

The use of brown sugar (experimental variant V2) is the best sweetener, even if it produce small changes in the intensity and tone of colour, it is protecting the reduced forms.

Honey (experimental variant V3) used as a sweetener has the highest absorbance of oxidized forms of heminic proteins, but the green tea environment loses transparency and clarity, both color intensity and color tone are affected and a slight oxidizing effect occurs. Honey produce the highest turbidity, which is a flaw of the final product, affecting customers visually.

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