

## VARIATION IN THE CONCENTRATION OF HEMIN PROTEIN IN SWEETENED BLACK TEA

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### Abstract

*Today, tea in the form of black or green tea, is the most widely consumed beverage in the world, next to water. About three billion kilograms of tea is produced and consumed yearly. Tea, made from the plant *Camellia sinensis* is consumed in different parts of the world. Of the tea produced worldwide, 78% is black tea (produced by fermentation), which is preferred by consumers in Western countries, 20% is green tea, which is commonly consumed in Asian countries, and 2% is Oolong tea (produced by partial fermentation).*

*The possible beneficial health effects of tea are being extensively investigated and have received a great deal of attention in recent times. Several studies show that black tea contains a number of compounds that reduce the risk of disease.*

*Heme proteins, that is, proteins containing one or several heme groups, are of immense importance for life on earth). They are involved in a wide range of fundamental cellular processes such as storage and transport of gaseous molecules (e.g. globins), energy conservation (photosynthetic and respiratory cytochromes), catalysis (e.g. detoxification enzymes such as catalase and cytochrome P450), and sensing changes in redox state or concentration of gaseous molecules.*

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

**Key words:** *cytochrome P450, black tea, heme proteins*

### INTRODUCTION

Black tea is the most consumed tea in the world. It has been shown to have a powerful antioxidant and blood lipid-lowering effect. The quality of black tea is determined by the following main factors: color, aroma and taste. The color changes of black tea are mainly associated with the levels of chlorophyll, carotenoids, theaflavins, teaflavins, teabrownins, flavonoids and other substances, with particularly significant variations in chlorophyll. The pigment that gives all plants their green color is called chlorophyll. Plants use chlorophyll to capture light for photosynthesis. The basic structure of

chlorophyll is a porphyrin ring similar to the heme in hemoglobin, but the atom at the center of chlorophyll is magnesium instead of iron. The long hydrocarbon tail (phytol) attached to the porphyrin ring makes chlorophyll soluble in fats and insoluble in water.

Hemin proteins represent photosynthetic and respiratory cytochromes involved in electron transfer, as well as oxygen-binding globins. An example of heme proteins is the very large group of cytochrome P450 enzymes. Naturally occurring porphyrins and related tetrapyrrole derivatives are involved in major life processes such as photosynthesis and the respiratory chain.

Chlorophyll, which is a partially reduced magnesium (II) porphyrin, is the main player in the conversion of solar energy into chemical energy in photosynthetic organisms (plants).

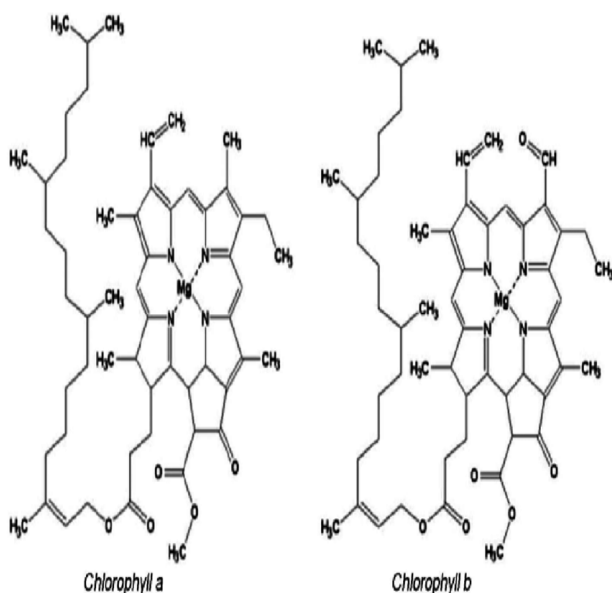


Figure 1. Molecular structure of chlorophyll a and chlorophyll b - according Asha Pai & Bipin Nair, 2015

## MATERIALS AND METHODS

The 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. 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 1 cm 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.

The single addition method allowed us to identify the range in which the highest values of specific molecular absorption spectra are obtained for black 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).

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

## RESULTS AND DISCUSSIONS

Analysing the graphs, we can see that the best sweetening variant is V2 (brown sugar) among the natural sweeteners and V5 characteristics are maintained by analysing graph 3. 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.

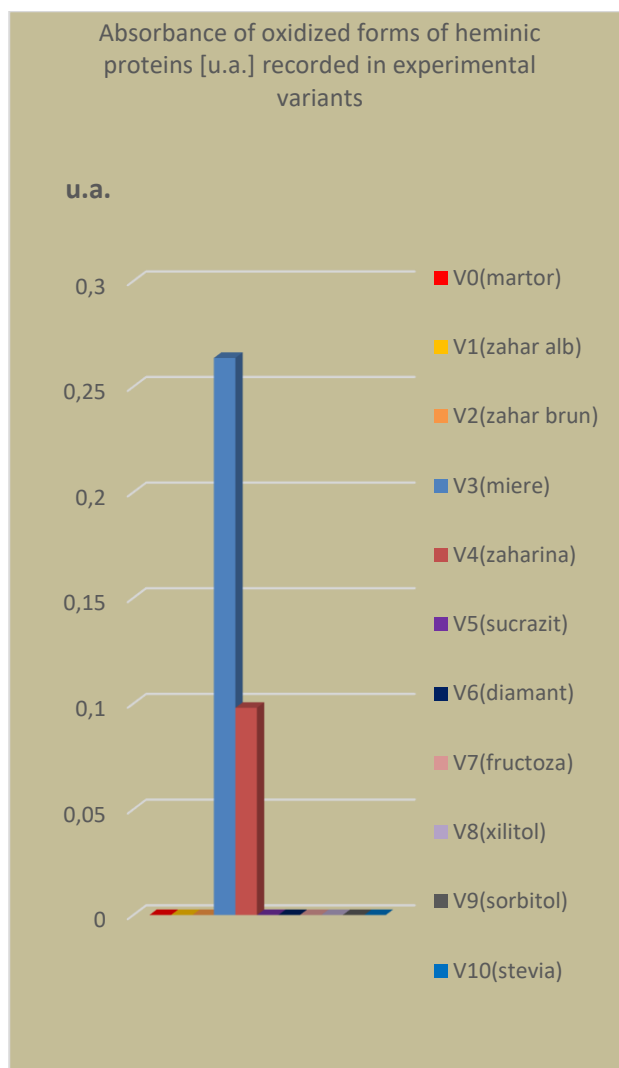


Figure 2. Absorbance of oxidized forms of heminic proteins [u.a.] recorded in experimental variants

(sucrose), which induce a high ratio. Graphs 3 and 4 define the best sweetening variant (V2 brown sugar) and we can see that these

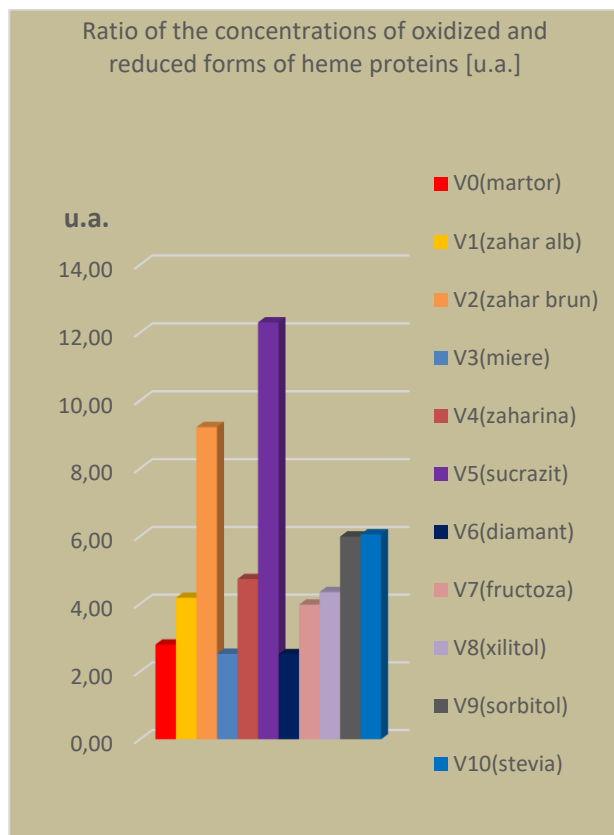


Figure 3. Ratio of the concentrations of oxidized and reduced forms of heme proteins [u.a.]

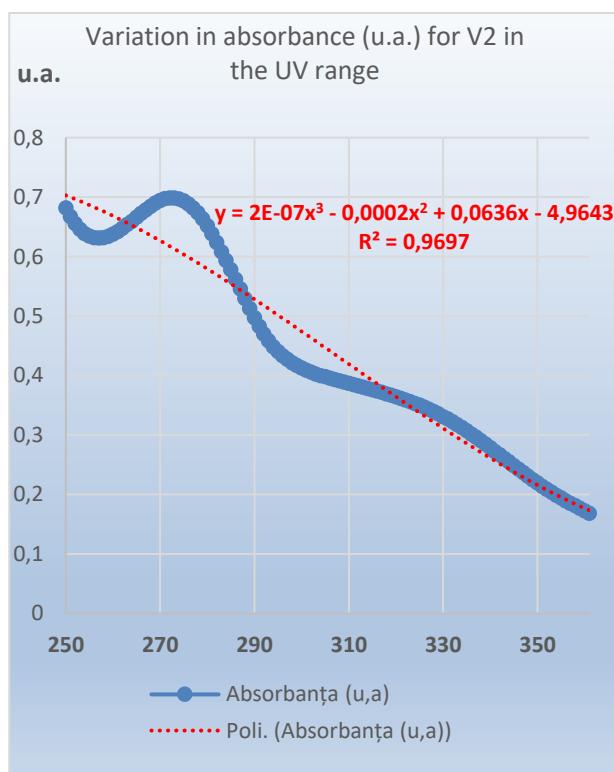


Figure 4. Variation in absorbance (u.a.) for V2 in the UV range

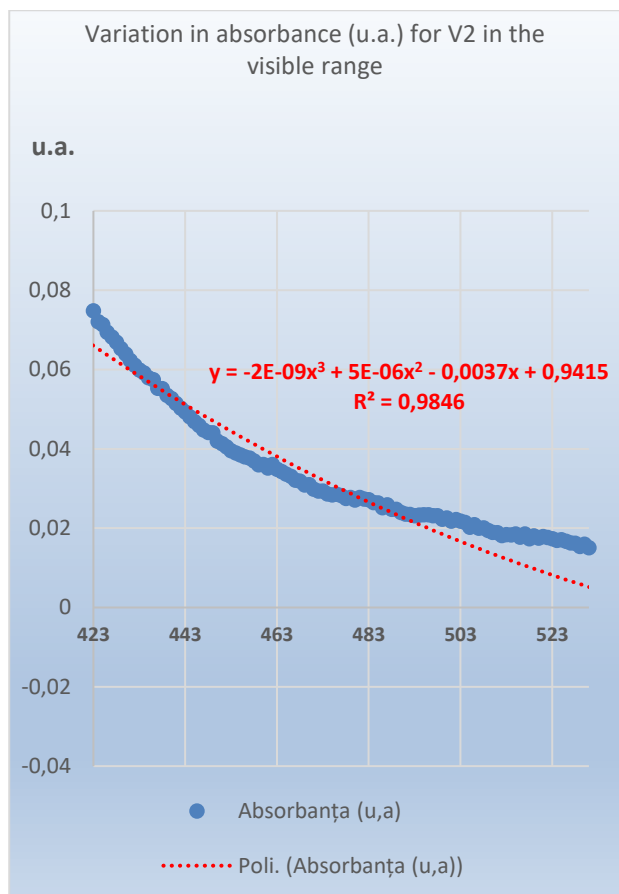


Figure 5. Variation in absorbance (u.a.) for V2 in the visible range

## CONCLUSIONS

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

Honey used as a sweetener in black tea induces a higher oxidizing state and brings about a change in the clarity of the tea. Sucrazite becomes more readily usable by consumers by buffering the active saccharin

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Figure 6. Experimental variants

nucleus, inducing sweet gulps under conditions of reduced oxidability. Brown sugar, even though it alters the color intensity and color tone, protects the reduced forms of which makes it a better alternative to white sugar. Honey and saccharin variants produce the highest turbidity, which can affect customers visually.

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