STUDY ON THE DEVELOPMENT OF A STRONG ANTIOXIDANT FUNCTIONAL FOOD BASED ON GREEN TEA

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Abstract

In recent years, there has been an increasing tendency amongst consumers to select functional food and beverages. This phenomenon can be attributed to the various properties of these foods, as well as the increasing demand among consumers for products that support a busy lifestyle and address health concerns.

Green tea is made from the leaves of the Camellia sinensis plant, which originates from Asia. The leaves are rich in the antioxidants chlorophyll and polyphenols due to their lack of oxidation and minimal processing. The following properties have been attributed to the green tea: beneficial effects on the circulatory system, improved heart function, prevention of atherosclerosis and obesity, and anti-carcinogenic, anti-inflammatory and antibacterial properties. The majority of these are attributable to the presence of biologically active substances in tea leaves, predominantly polyphenolic compounds, as well as alkaloids (theobromine, theophylline and caffeine) and theanine. Green tea contains a large number of NAD- and FMN-dependent enzymes and it is essential for food engineers and innovative product developers to study how these enzymes behave under tea additive conditions. The aim of this paper is to successfully find which sweeteners have the minimum impact on the antioxidant activity in a sweetened green tea functional beverage.

Key words**: **functional food, antioxidants, sweeteners, green tea, oxidoreductases

INTRODUCTION

Recent shifts in consumer food consumption behaviour have been observed, with an increasing focus on health and disease prevention. This has resulted in heightened investment in research and development of functional foods. These foods, in their conventional form, offer specific health benefits beyond basic nutrition, appealing to individuals seeking to enhance their overall health and well-being.

The increased consumption of functional plant-based foods and beverages can be attributed to changes in socio-economic status, consumer preferences and innovative technologies. For instance, the population structure has undergone persistent shifts towards increased life expectancy. This phenomenon has had a consequential impact on the necessity for consumers to consume functional foods, with the aim of promoting or preserving a full and active lifestyle.

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carcinogenic, anti-inflammatory and antibacterial properties. The majority of these are attributable to the presence of biologically active substances in tea leaves, predominantly polyphenolic compounds, as well as alkaloids (theobromine, theophylline and caffeine) and theanine.

Green tea contains a large number of NAD- and FMN-dependent enzymes and it is essential for food engineers and innovative product developers to study how these enzymes behave under tea additive conditions.

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. 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.

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).

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).

In certain liquid foods, these mechanisms have been observed to be susceptible to disruption by specific food additives, including sweeteners, preservatives, and colourants. Sweeteners have been demonstrated to exert an oxidative effect on these redox systems, with the potential to considerably diminish the antioxidant capacity of foods. The presence of sweeteners in green tea has been demonstrated to reduce the concentration of reduced active forms of certain oxidoreductases, with a particular effect on metal-flavoprotein enzymes.

The aim of this paper is to successfully find which sweeteners have the minimum impact on the antioxidant activity in a sweetened green tea functional beverage.

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.

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. The resulting variants are listed below:

V0 – an unsweetened green tea variant

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.
Before processing, the green tea used in the design of this type of functional food was tested by Atomic Absorption Spectroscopy (AAS) to rule out the presence of heavy metals. Analysis of the changes in the molecular absorption spectra curves was carried out using UV-VIS and NIR (Near-Infrared) spectrometry.
For calibration curves specific to the main green tea biocompounds, the Single Addition Method, Pure Analysis substances (from Merck) and Certified Reference Materials (CRMs) were used. The change in the concentrations of oxidized and reduced forms of some bio-compounds with high antioxidant capacity in green tea was recorded by using electro-chemical techniques. For this study, the concentrations of oxidized and reduced forms of some NAD- and FMN-dependent oxidoreductases were also carefully analysed.
This study utilized a number of sustainable reagent-free analytical techniques including UV-Vis spectrophotometry, AAS, NIR and electrochemical titrations.

Fig. 1 Experimental variants

RESULTS AND DISCUSSIONS

The experiments carried out in this study have proved the existence of a strong correlation between the nature of the sweeteners used and the changes produced in the antioxidant capacity of some valuable bio-compounds in green tea. These are very helpful in establishing diets associating green tea with certain foods (high in synthetic food additives) or drugs (synthetic, especially of the type of antidepressants used in children with ADHD).
The selection of the additives used in the sweetening operation must take into account the preservation of the antioxidant character of the finished product (maintaining a high level of reduced forms).
During the stages of obtaining innovative functional food product from green tea, it is recommended to monitor the activity of oxidoreductases 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 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 functional food.

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	-

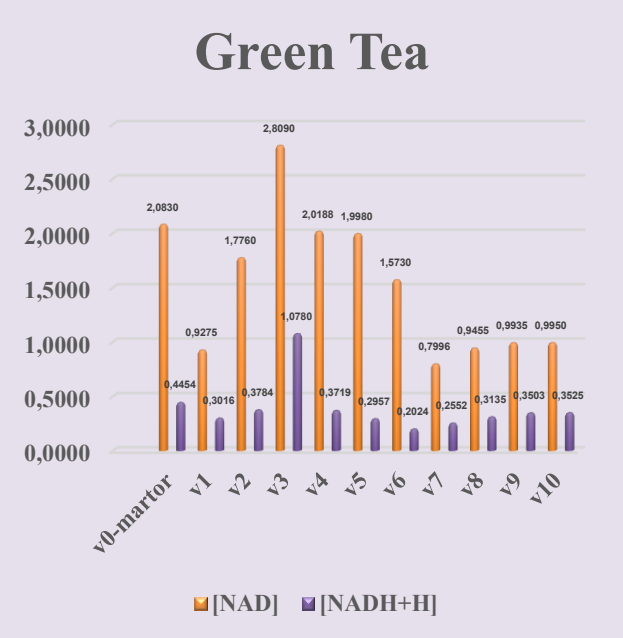

Fig. 2 The NAD and NADH+H concentration for experimental variants

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

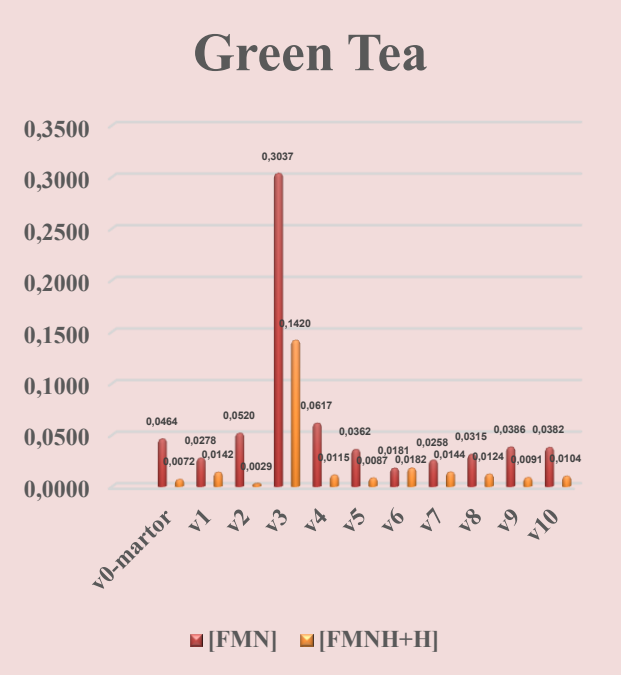

Fig. 3 The FMN and FMNH+H concentration for experimental variants

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

White sugar (V1) induces changes in the oxidoreductases present in green tea, decreasing their presence.

The use of brown sugar (V2) produce minimum alterations in the activity of oxidoreductases, maintaining the levels of NAD, NADH+H, FMN and FMNH+H closer to the unsweetened version (V0).

Honey can create the strongest oxidation on the surface of the sweetened tea, changing also the redox potential value in these variants. The influence of honey on the characteristic pigments and thiophilic-type alkaloids in green tea varies according to the origin of the green tea and the temperature at which the sweetener is added.

Saccharin (V4) 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 (V5) is added to the medium, the oxidation effects

are much less as the coloured pigments are better protected.

The use of fructose (V7), xylitol (V8), sorbitol (V9) and stevia (V10) produces similar changes in the composition of sweetened green tea, reducing its antioxidant character.

CONCLUSIONS

Several clear conclusions emerge from the laboratory analysis:

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).

Honey produce the highest turbidity, which is a flaw of the final product, affecting customers visually. The use of white sugar (V1) and Diamond (V6) results in low concentration of oxidoreductases. Fructose (V7), xylitol (8), sorbitol (V9) and stevia (V10) present lower antioxidant activity than the unsweetened variant (V0).

The optimal sweeteners are those that produce minimal changes in the chemical composition while retaining all the beneficial characteristics of green tea. These are brown sugar (V2) and saccharine (V4).

The results obtained from this study will undoubtedly lead to the optimization of these additivities techniques. The best experimental variant was the one that produced the least redox imbalances. The study highlighted the possibility of selecting the best sweetener for this type of tea, of optimizing certain technological operations and manufacturing recipes, and especially the effects produced by association with certain foodstuffs or medicines.

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