RESEARCHES REGARDING THE CHANGES OF THE NAD AND FMN COENZYMES STATE FROM THE LEMON JUICE AFTER SWEETENING TASK

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ABSTRACT

The work paper is a side of complex study regarding the effects of natural sweeteners on the lot of liquid foods obtained by the agricultural products [8].

Follow the increased consume of the lemon juice in present time it is necessary to knowing the effects of sweetening task on the consumers' human bodies for prove and promote the best sweetener for this juice.

The lemon juice experimental variants were prepared and sweetened with some of most used sweeteners for Romania and the changes of the state of juice were registered. The monitoring can be use for promote the healthy sweeteners and for establish the best time of preserve for this juice.

INTRODUCTION

The origin of the lemon is unknown, though lemons are thought to have first grown in Assam (a region in northeast India), northern Burma or China.[4] A study of the genetic origin of the lemon reported it to be hybrid between bitter orange (sour orange) and citron[5]. Lemons entered Europe near southern Italy no later than the first century AD, during the time of Ancient Rome.[4] However, they were not widely cultivated. They were later introduced to Persia and then to Iraq and Egypt around 700 AD.[1] The lemon was first recorded in literature in a 10th-century Arabic treatise on farming, and was also used as an ornamental plant in early Islamic gardens.[6] It was distributed widely throughout the Arab world and the Mediterranean region between 1000 and 1150.[6]

Lemons are a rich source of vitamin C, providing 64% of the Daily Value in a 100 g serving [7].. Other essential nutrients, however, have insignificant content. Lemons contain numerous phytochemicals, including polyphenols and terpenes. [7] As with other citrus fruits, they have significant concentrations of citric acid (about 47 g/l in juice). [7]

Lemon juice combined with papaya juice is a so-called "digestant" and is excellent for digestive problems [9].

The study of redox potential, of cofactors of oxidoreductase from lemon juice can offer the solution of optimal sweetening receipt with low cost of reagent.

In this work paper it is proved the link between redox status (characterized by coenzymes of oxidoreductases from lemon juice) and the finding the best natural sweeteners for this juice.

Nicotinamide adenine dinucleotide, abbreviated NAD^+, is a coenzyme found in all living cells [2]. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups: with one nucleotide containing an adenosine ring, and the other containing nicotinamide. In metabolism, NAD^+ is involved in redox reactions, carrying electrons from one reaction to another [1]. The coenzyme is therefore found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, this reaction forms NADH, which can then be used as a reducing agent to donate electrons [1]. These electron transfer reactions are the main function of NAD^+ [2].

Flavin mononucleotide (FMN), or riboflavin-5′-phosphate, is produced from riboflavin (vitamin B_2) by the enzyme riboflavin kinase and functions as prosthetic group of various oxidoreductases including NADH dehydrogenase. During catalytic cycle, the reversible interconversion of oxidized (FMN), semiquinone ($FMNH^{\bullet}$) and reduced ($FMNH_2$) forms occurs. FMN is a stronger oxidizing agent than NAD and is particularly useful because it can take part in both one and two electron transfers [3, 5].

MATERIAL AND METHOD

For obtain the witness variant (unsweetened) were used lemon fruits (Sorrento type) and these were pressed and adequate separated.

One lemon variety (Sorrento), that have been mainly used for lemon juice concentrate production in Romania (and limoncello in Italy – the original country), was the base for to obtained the fruit juice for this experiment.

After storing overnight at 4°C, the fruits were processed to lemon juice. Lemon juice was prepared from approximately 2 kg lots of lemons. After sorting and washing these fruits were ground and the juice extracted using a lab-scale fruit juice extractor (Progress Juice Extractor - experimental variant). The obtained pressed drink was decanted and filtered (through a porous cellulosed material). After the filtration task the lemon juice was centrifugal separate into a performance centrifuge “Sygma” type, at a 5000 rot/min during 4 minutes. For to quantify into the changed juice the NAD^+ and $NADH+H^+$ content and the FMN^+ and $FMNH+H^+$ content after sweating task with natural and synthetic’s sweeteners it is constituted ten experimental variants.

After the separation it had been picked a median sample of 50 ml pear drink that was diluted; this variant being the unsweetened reference one.

From unsweetened variant of natural lemon juice V_1 it is obtained through sweetness task the follow experimental variants:

V1- unsweetened natural lemon juice (reference sample),
V2- natural lemon juice + white sugar,
V3- natural lemon juice + brown sugar,
V4- natural lemon juice + stevioside,
V5- natural lemon juice +fructose,
V6- synthetic lemon juice
V7- synthetic lemon juice + white sugar,
V8- p- synthetic lemon juice + brown sugar,
V9- synthetic lemon juice + stevioside,
V10 - synthetic lemon juice + fructose.

The used sugar for experimental variants has proved a concentration of 2.5g/50mL natural lemon juice concentration. The stevioside (green sugar) has proved in to V_4 on 25 mg/50mL natural lemon juice and the longer solvated time. For obtain the V_9 it is used stevioside in 25mg/50mL synthetic lemon juice content and the solvated time was increased.

Steviol glycoside is a more precise term for a group of intensely sweet compound extracted and purified from *S.rebaudiana*. Stevioside and rebaudioside A are the predominant steviol glycosides found in *S. Rebaudiana* [11]. Commercial interest in steviol glycoside sweeteners has been high for a long time [10]. The steviosides for the V4 and V9 were from the Vitalia K Pharma provider and were absolutely natural (obtained trough extraction).

The fructose for V5 and V10 was natural sweetener and was produced by NATEX and has 99.9% purity.

The experimental samples were spectrophotometer to a digital performances spectrophotometer UV-Vis “Unicam 2” type (with the spectral band width of 1mm) in the

nearly UV range (190-400 nm), the visible range (400-700 nm) and nearly IR range (700-1100 nm). Using the soft, the Deuterium lamp was automatically changed with the Tungsten at 325 nm. The used cuvettes were from quartz and proved 4.5mL capacity and 10nm width. For decreasing the limits of errors, the obtained results were replayed in to auto- re-tracking and save in to files .qnt format and convert with the soft Visio ver.2.0.

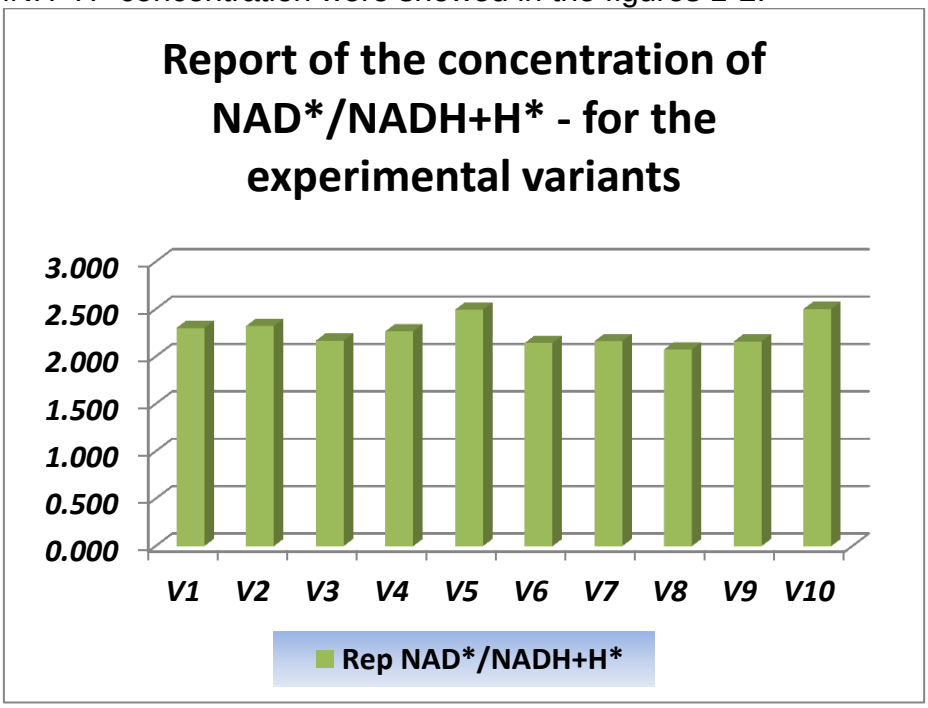
Both NAD^+ and NADH absorb strongly in the ultraviolet due to the adenine base. The peak absorption of NAD^+ is at a wavelength of 259 nanometers (nm), with an extinction coefficient of 16,900 $M^{-1}cm^{-1}$. NADH also absorbs at higher wavelengths, with a second peak in UV absorption at 339 nm with an extinction coefficient of 6,220 $M^{-1}cm^{-1}$. This difference in the ultraviolet absorption spectra between the oxidized and reduced forms of the coenzymes at higher wavelengths makes it simple to measure the conversion of one to another in enzyme assays – by measuring the amount of UV absorption at 340 nm using a spectrophotometer [2].

The FMN and $FMNH+H^+$ contents in to pear juice were determined through use the spectroscopy in to Visible range (400-700 nm) and the maximal molecular absorption spectra were determined by only add pure analysis substances method.

During the analysis for the experimental variants it has been taken all the treatments, for having a minimal temperature changes at the maxim limit of the interpedently substances influence, the assure the optimal needed conditions for a average analytical errors limits. For to interpret of the results it has been used from the utilitarian packet MS Office 2003: MS Word 2003 and MS Excel 2003. The statistical analysis for data obtaining has been effectuated with the SPSS11.0- statistical software for Windows.

RESULTS AND DISCUSSIONS

It has been controlled experimentally the influence of the edulcorants added in the lemon juice, evident the Absorption in the nearly UV range, Vis and near IR range too. The obtained results after determination of concentration of NAD, $NADH+H^+$ (both form), and the FMN, $FMNH+H^+$ concentration were showed in the figures 1-2.



The greatest content of NAD, NADH+H+ (both form) were registered at the experimental variants of sweetened lemon juice that use the fructose like as sweeteners; in this case use the fructose conduct to increase of the anaerobic oxidoreductase activity.

The experimental variant that use sugar for sweetening the natural lemon juice (V2) present the NAD concentration close to witness.
The experimental variant that use sugar for sweetening the natural lemon juice (V2) present the NAD concentration close to witness (figure 1).
The best reports of coenzyme of anaerobic oxidoreductases (NAD) were registered to the variants that use brown sugar or stevioside like as sweeteners for natural juice lemon (2.168 registered value for V3 and 2.267 registered values for V4).

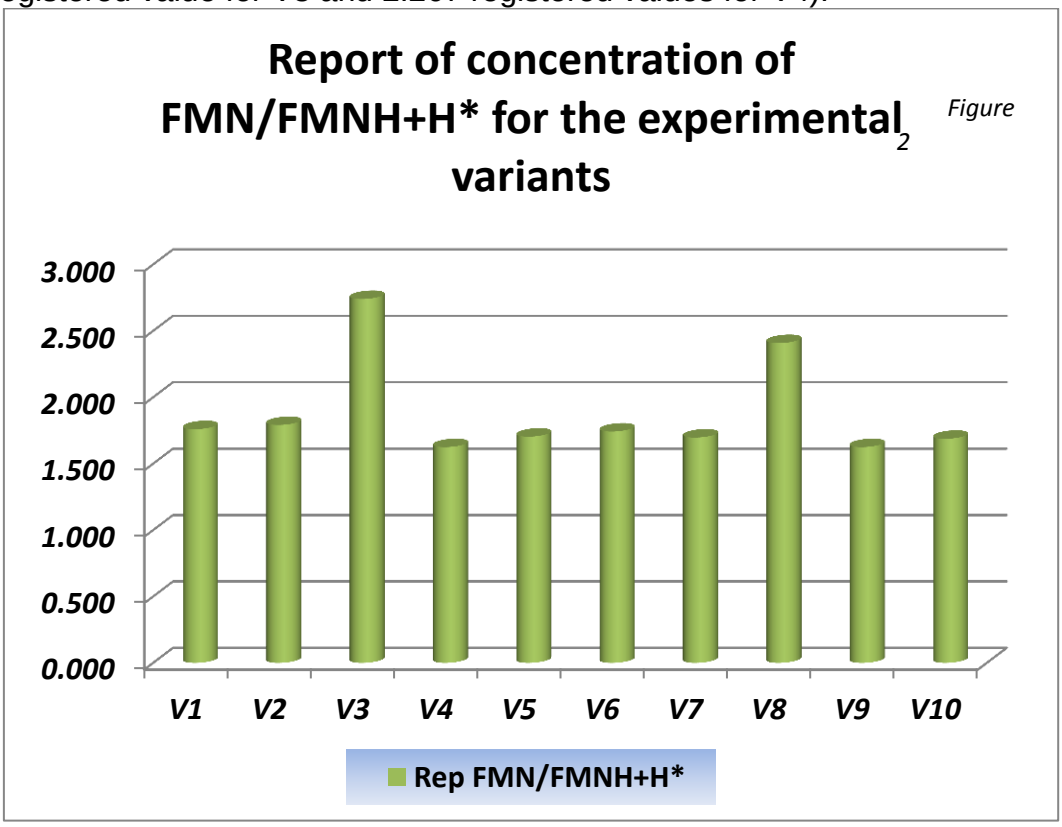


Figure 2

The fructose prove increase of oxidative stress to natural lemon juice (V5) and to synthetic lemon juice (V10 – the biggest oxidative report for NAD, 2.508 -in registered value, figure 1). The caloric effect of brown sugar added in natural lemon juice (V3) and in synthetic lemon juice (V8) was correlated with the biggest value of concentration of reduced and oxidized NAD forms for these variants (fig.1). The experimental variants that used the green sugar (stevioside in V4 and in V9) are registered the lowest value of NADH report than the witness. The stevioside added in to V9 are influenced the NAD smallest report (figure 1).

The caloric effect of brown sugar added in natural lemon juice (V3) and in synthetic lemon juice (V8) was correlated with the biggest value of concentration of reduced and oxidized FMN forms for these variants (fig.2).

The experimental variants that used the green sugar (stevioside in V4 and in V9) are registered the lowest value of report FMN content than the witness. The stevioside added in to V9 are influenced the report of FMN in smallest content (figure 2).

The best reports of coenzyme of aerobic oxidoreductases (FMN) were registered to the variants that use stevioside like as sweeteners for natural juice lemon (1.625 registered values for V4) (figure 2)

The best reports of coenzyme of aerobic oxidoreductases (FMN) were registered to the variants that use stevioside like as sweeteners for synthetic juice lemon (1.625 registered values for V9 – same value for V4) (figure 2)

CONCLUSIONS

The optimal sweetening variant for pear juice through new method-with low costs can be used through the study of changed main compounds concentration. For this study it can use the optical and statistic analysis, with lower consumption of reagent.

- The method of analysis that use the UV VIS spectrometry can be a good and cheaper method of analyse than HPLC methods for determinate the concentration and effect of sweeteners, the UV- VIS optical methods can be used for to determinate the best edulcorants for the natural pear juice and can be complete with FTIR spectrometry (for analysis the any isomers derivate from compounds of base);

The caloric effect of brown sugar added in natural lemon juice (V3) and in synthetic lemon juice (V8) was correlated with the biggest value of concentration of reduced and oxidized NAD and FMN forms for these variants.

The experimental variants that used the green sugar (stevioside in V4 and in V9) are registered the lowest value of NAD and FMN report than the witness. These steviosides added in to V9 are influenced the NAD and FMN smallest report.

The best reports of coenzyme of aerobic oxidoreductases (FMN) were registered to the variants that use stevioside like as sweeteners for natural or synthetic juice lemon (1.625 registered values for V4 and V9).

This method can be successful used in determinate of the best report of oxidative status for the lemon juice and can recommended the best way to processing and/or preserving this important resource of natural vitamin.

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