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Original scientific paper

The amount of active hydrogen in selected juices and teas on the Slovenian market

Borut **POLJŠAK**[1]

ABSTRACT:

The ageing process is influenced by increased oxidative stress, which causes damage to cellular components, such as DNA, proteins and lipids. Many studies claim that use of antioxidant might prevent intracellular oxidative damage formation and thus decrease the incidence of age related diseases (cancer, cardiovascular diseases, cataract...). The level of endogenous antioxidants cannot be deliberately increased. However, the level of exogenous antioxidants intake can be increased by consuming more fruit, vegetables or their juices, which are a rich source of many different antioxidants. Today consumers are aware of a healthy way of living, thus the antioxidant status of a product can be important information in the decision of purchase.

We measured the antioxidant status in selected fruit juices and teas. Since each exogenous antioxidant (vitamin C, E, beta carotene) prevents oxidation or quenches free radicals by donating one electron or H^-. For this reason the amount of active H (H^-) was determined as an indicator of the total antioxidant potential. Besides, the intake of H^- with natural products is better than with synthetic ones, because fruits and vegetables contain many different antioxidants which can regenerate each other. It was concluded that the total antioxidant potential, measured as the relative amount of H^- (partial H pressure), is a better indicator of the antioxidant status of a specific food product, than the determination of only one specific antioxidant, eg. vitamin C, E or beta carotene. The results indicate significant differences in the rH level among different drinks. The highest value of active hydrogen was determined in tomato juice, red grapes juice, carrot, blackcurrant, nectar of strawberry, apple and grapes, orange juice, pineapple juice, sour cherry nectar and blueberry tea. There is much active hydrogen also in the pear nectar, apricot nectar, peach nectar, banana nectar, nectar made of seven juices and five mashes, but these are with added synthetic antioxidants (ascorbic acid) that contribute to the increased value of active hydrogen. The antioxidant potential of teas is lower than the antioxidant potential of the majority of juices. However, the portion of active hydrogen is in all selected beverages is much larger than in potable water.

KEY WORDS:

Active H, Antioxidants, Oxidative stress, Juices, Teas

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[1] Borut Poljšak
University of Ljubljana,
University College of Health Studies,
Department of Sanitary Engineering
Poljanska 26a, SI - 1000 Ljubljana,
Slovenia
E-mail: borut.poljsak@vsz.uni-lj.si

INTRODUCTION

Currently, many researches are being conducted on vitamins mainly in the two fields: in the field of the daily needs and recommended daily intake of vitamins and minerals, and in the field of ageing prevention, oxidative stress, cardiovascular diseases and cancer [1].

Antioxidants are substances that slower or prevent the oxidation of important cellular components in various ways. After the reaction with the radicals antioxidants are transformed into more stable products or less noxious radicals, which are not reactive enough to cause free radical mediated chain reactions. Mostly, after the job is finished, antioxidants are worn-out. Some (ex. vitamin C and vitamin E) renew and regenerate in the oxidation-reduction processes.

Results of basic researches discovered an important role of vitamins in the pathophysiology of diseases connected with oxidative stress [2,3] such as: arteriosclerosis, hypertension, cataract, rheumatic arthritis, malign diseases, Parkinson's and Azheimer's diseases [4-6]. Epidemiological studies determined a lower rate of cancer, heart and veinal diseases between persons that with food consumed higher quantities of vitamins C, E and β-carotene. An increase of the serumal level of these vitamins was noticed in these persons [7-9]. Until now, researches ascertained a lower break out of cancer, heart and veinal diseases only between people that consume enough quantities of fruit and vegetables, but not between people that consume supplements of vitamins [10]. The consumption of fruit and vegetables lowers the formation of free radicals in the body and thus the induced oxidative lesions of the DNA, meanwhile many studies show that only supplements of vitamins C, E and beta carotene do not lower DNA lesions [11-15]. Researches confirm that is best if the protective substances (antioxidants and vitamins) originate from food [16]. Positive effects of the protective substances that originate from food are greater because of the synergic activity between individual antioxidant substances [16], nutritional fibrin and secondary vegetal substances in food, mainly in vegetables and fruit. Vitamin E can regenerate due to vitamin C, the later due to glutathione [16,6]. If such regeneration does not occur, the oxidized versions of the previously mentioned antioxidants can cause unwanted pro-oxidative effects and damage in cells [6,16]. These unwanted effects can potentially occur with the excessive intake of an individual antioxidant.

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rH – indicator of hydrogen content

Every endogenous antioxidant neutralizes one free radical by donating one electron or H^- (Figure 1). With that purpose we selected, as an indicator of antioxidant potential of drinks, the indicator of hydrogen content in a biological environment expressed as partial pressure of hydrogen (rH or rH_2). rH is the "absolute indicator of the reductive potential" of a substance [17]. It shows the quantity of ions of active hydrogen in solutions, of either organic or inorganic origin. Levels of rH are mostly between 0 and 42. Level 42 means dissolved oxygen saturation rate, when the level of rH is 1, it means that the substance is rich with hy-

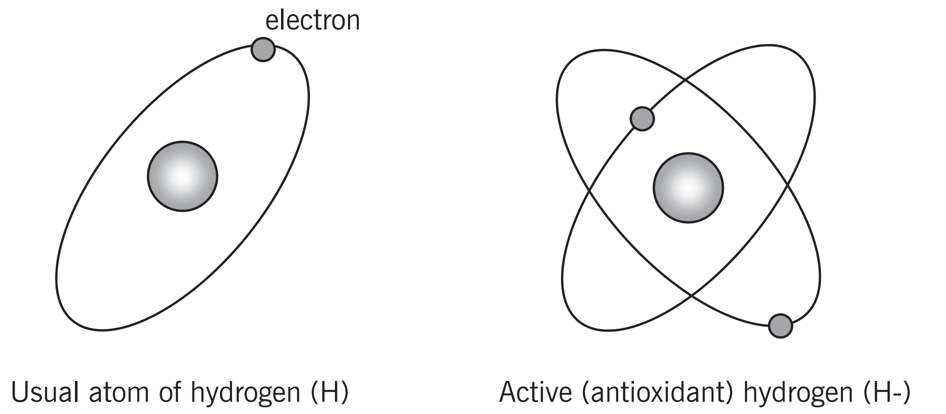

Figure 1.

Active hydrogen. Hydrogen is the smallest existing atom. Usually includes one proton that composes the core and one electron that circulates around it. In particular circumstances the atom is capable of retaining an extra electron. In literature it is called "active" or "anion" hydrogen, because it is the base of all antioxidant processes in the body and it is also called "antioxidant" hydrogen.

drogen. At this level there is no free oxygen left [17]. It is possible to obtain lower or even negative levels of rH, which represents even greater concentrations of active hydrogen. The maximum level is 28, what is above 28 means oxidization (predomination of oxidizing agents), while levels below 28 mean reduction (predomination of reducing agents).

Biological environment must be in a reduced form, thus levels of rH in biological liquids must contain greater values of hydrogen than oxygen, therefore the level of rH must be considerably below 28. The increase of rH of one unit namely means a 10 times lowering of the quantity of active hydrogen ions [17].

The purpose of this research is to determine the share of active hydrogen in natural food sources-fruit juices and teas. For these reason a relatively simple, but sensitive method that quantifies the antioxidant capacity of beverages was introduced.

METHODS

For the measurement of oxidation-reduction potential (ORP) and pH levels the simultaneous use of three instruments was performed: namely Inolab WTW pH meter, HACH Sension pH meter, HACH Sension ORP meter and Greisinger electronic ORP meter. All measurements were performed in a 100 mL cup, previously mixed, at room temperature 25 °C. The final measured levels of pH and ORP were read in mV.

The criterion for the reaction capability of a compound are oxidization/reduction potentials in mV. Reduction potential (also known as redox potential, oxidation/reduction potential or ORP) is the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. pH of the solution is the criterion of concentration of free positive hydrogen ions in the solution. The use of rH gives a hydrogen proton-unbiased look at the absolute reducing potential of a compound, eliminating the effect of pH in the ORP measurement. It is a true indi-

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cation of a compounds reduction potential capacity. The shifts in rH can be used to quantify the reducing ability and energy reserves of the compound. The rH level is the criterion for the state of reduction or oxidation in which is the compound, it is also the indicator of the probability that the compound will react with the free radical. The direct use of pH and reduction potential measurements (ORP) gives an indication of the probability of a compound to act as an antioxidant [21,22].

Nerst equation and rH

Because of the interaction of protons at the changes of pH oxidation-reduction potential may be biased by the pH and vice versa. For this reason the variation of Nernst equation (Equation 1) was used, which is an effective way for measuring the reductive potential of a compound, which is given by the level of rH. This is the logarithmic value and is the criterion for absolute reductive potential.

$$E_h = 1{,}23 - \frac{RT}{F}\,\mathrm{pH} - \frac{RT}{4F}\ln\frac{1}{P_0} \qquad (1)$$

E_h in the equation is the measured reductive potential (mV), F is the Faraday constant (the charge per a mole of electrons), equal to $9.6485309 \cdot 10^4$ C mol^{-1}, R is the universal gas constant, equal to 8.314510 $J \cdot K^{-1} \cdot mol^{-1}$ and T is the temperature in Kelvin. (Kelvin = 273.15 °C). The value 1.23 in the equation is the potential of oxygen at one atmosphere (101.235 kPa) 1.23 V higher than in the compound at the same pH. The level of rH is explicitly defined as the negativelogarithm of oxygen pressure, P_o (equation 2).

$$\mathrm{rH} = \log P_0 \qquad (2)$$

rH is the “absolute indicator of the reductive potential” of a substance [17]. It shows then concentration of active hydrogen ions, rH can be determined indirectly with the determination of ORP and pH. The formula for its reckon was already discovered in 1923 by Clark (18) (remodelled Nernsto’s equation), but only in later years it is gaining full value at studying processes in living beings. Basically it is a complicated logarithmic formula, but in practice (for measurements at 25 degrees Celsius) a simplified formula is used (equation 3):

$$\mathrm{rH} = \frac{(ORP + 204)}{30} + 2 \cdot \mathrm{pH} \qquad (3)$$

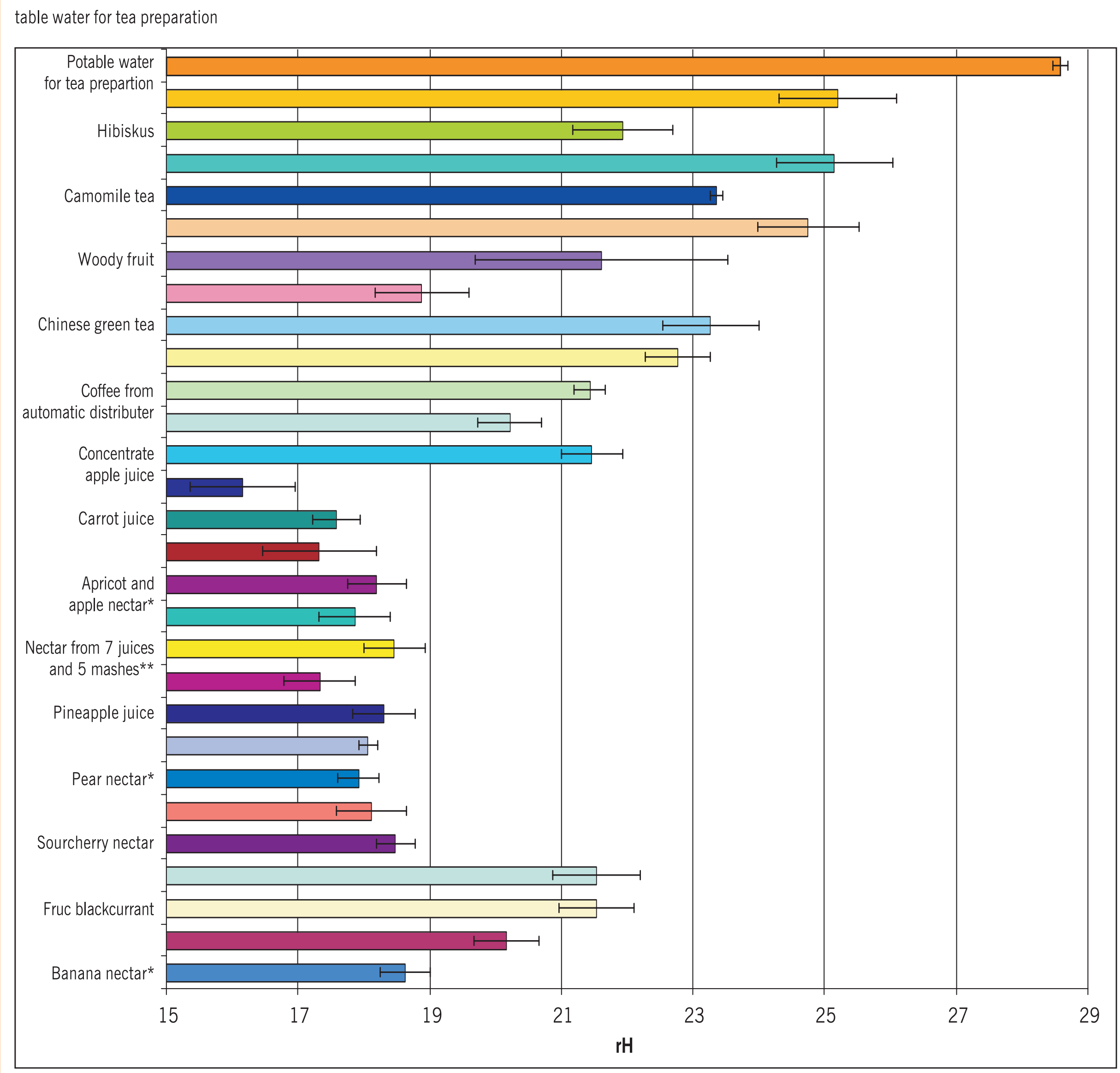
RESULTS

18 fruit juices (with different fruit/vegetable intake) and 9 teas were randomly chosen from the supermarket. Coffee was made from a public automatic distributor. In all beverages the portion of active hydrogen was determined.

The portion of active hydrogen is in all selected beverages much larger than in potable water, which is between rH=29 and rH=39. This means that all beverages have an increased value of antioxidants comparing to potable water. However the shares of active hydrogen vary significantly between singular beverages (Figure 2). This way we determined the highest value of active hydrogen in tomato juice, red grapes juice, carrot, blackcurrant, nectar of strawberry, apple and grapes, orange juice, pineapple juice, sour cherry nectar and blueberry tea. There is active hydrogen also in the pear nectar, apricot nectar, peach nectar, banana nectar, nectar made of seven juices and five mashes, but these are with added synthetic antioxidants (ascorbic acid) that contribute to the increased value of active hydrogen. The antioxidant potential of teas is lower than the antioxidant potential of the majority of juices, most likely because of different temperatures at preparation process (90 °C vs. pasteurisation). When preparing fruit juices, the pasteurization process is used for the process of heat treatment, with which pathogenic

Figure 2.

Schematic view of the portion of active hydrogen in the chosen beverages.



* added ascorbic acid (vitamin C) ** added various vitamins

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micro organisms are destroyed and enzymes are inactivated for a short time at the temperature below 100 °C.

The level of rH is presented in a logarithmic scale, that means that both apple juice and coffee (both rH = 21.5) have approximately the same number of electrons in the equal unit of volume of the drink. One glass of coffee (rH = 21.5) is, for the share of H^-, almost equivalent to 10 glasses of green tea (rH = 22.7). One glass of sour cherry nectar is, for the share of electrons, approximately equivalent to 100 glasses of blueberry nectar. All juices and teas have a considerably higher share of H^- than the ordinary water from the waterworks.

DISCUSSION AND CONCLUSIONS

In current times due to our lifestyle, industrial pollution, climatic changes, overpopulation, countless vehicles, increased UV-radiation because of ozone layer depletion, cigarette smoke, infections, unhealthy way of living – human population is constantly exposed to free radical formation and all this causes increased “oxidative stress” in our bodies and related diseases in the population. The most frequent cause for oxidative stress formation is certainly cigarette smoke, which causes degenerative diseases and premature death.

A healthy adult person does not need additional vitamin and mineral supplements if he eats varied diverse food with a sufficient energy intake. However present ways of processing food led to a point where food products are impoverished of minerals and vitamins. Due to intense farming, soil is lacking minerals, because of transport fruit is picked up unripe, however it is known that antioxidants synthesize only at ripening [6], because plants use them to protect from the environmental effects (UV-radiation), diseases, pest and other stressful factors [23]. Furthermore, fruit is sprayed with pesticides that can extinguish the beneficent characteristics of protective substances in fruit. Unsuitable mechanical preparation and heat treatment can greatly decrease the nutritive value of a food product and affect the change of antioxidant potential of food [24]. The majority of Slovenians do not daily consume five portions of fruit and vegetables. Data from the research “Risk factors for contagious diseases between adult residents of Slovenia“ [25] show that 31.6 % of adults in Slovenia do not eat vegetables daily and 43.0 % do not eat fruit daily. 21.9 % of the inquired in this research, eat vegetables several times a day and 31.1 % eat fruit several times a day.

A healthy adult person does not need additional vitamin and mineral supplements if he eats varied diverse food with a sufficient energy intake.

All this states are in favour of additional intake of minerals and vitamins, which can be accomplished with increased regular consumption of fresh and raw fruit and vegetables and their juices. It has to be realized that the use of synthetic vitamin supplements is not an alternative to regular consumption of fruit and vegetables. Fruit contains thousands of compounds with unknown effects on health. Probably many antioxidants are still undiscovered, furthermore the combination of antioxidants in fruit and vegetables is optimal, as it causes their reciprocal regeneration and consecutively intensifies their defence from free radicals. Compared to vitamin and mineral supplements the advantage of consuming natural food products is in their complex and balanced content of macro and micro nutrients that enable a harmonious effect of separate active substances and consecutively less probabilities for over consumption of active components [24].

Since endogenous free radical formation cannot be deliberately increased, all we can do is increase the level of exogenous antioxidant protection of our bodies by intake of antioxidants from food, namely fruit, vegetable and their juices and teas. In the present analyze we confirmed increased amount of active H in all drinks tested as compared to potable water; with great difference between particular type of drink. The highest value of active hydrogen was in tomato juice, red grapes juice, carrot, blackcurrant, nectar of strawberry, apple and grapes, orange juice, pineapple juice, sour cherry nectar and blueberry tea. However, the greatest amount of active H was still in the two freshly prepared juices tested – orange juice (rH = 13.8) and pineapple juice (rH = 16.7) indicating that by industrial juice preparation and during the storage some antioxidant potential is lost.

It is evident that the antioxidant potential of a drink is useful and important information for consumers and should be written on the product label.

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