Antioxidant capacity of camu camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh] pulp*

Antioxidative Kapazität des Fruchtfleisches der Camu-Camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh]

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Summary

Camu camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh] is a shrub from the Myrtaceae family. It grows naturally in the Amazonian basin. The fruit has the highest content of natural vitamin C known (about 1000-3000 mg/100 g). In comparison with other fruits, camu camu presents outstanding antioxidant features. The Total Oxidant Scavenging Capacity (TOSC) assay was chosen for its examination because it is based upon the reactive oxygen species peroxyl radical, hydroxyl radical and peroxynitrite which are of importance in the human metabolism. Camu camu samples from two different sources exhibited almost identical antioxidant capacities against peroxyl radicals and peroxynitrite – the highest values in comparison with other juices, until now studied. The inhibitory effect of the two samples against hydroxyl radicals differed substantially. One of them presented an effect in the same range as blueberry and apple juice, the other one was notably more active. Studies on the reason for that partially equal and partially different reactivity of both samples are initiated. Polyphenolic compounds that are obviously partially responsible for the overall antioxidant capacity were identified by HPLC-MS as flavonolglycosides.

Keywords:
Myrciaria dubia, camu camu, vitamin C, TOSC assay, antioxidants, polyphenols.

Zusammenfassung

Camu-Camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh] ist ein strauchartiges Gewächs aus der Familie der Myrtaceen. Die Früchte haben den höchsten in der Natur bekannten Vitamin C-Gehalt (1000-3000 mg/100 g). Im Vergleich mit anderen Fruchtsäften zeigt Camu-Camu-Saft eine herausragende antioxidative Kapazität. Für deren Untersuchung wurde der „Total Oxidant Scavenging Capacity (TOSC)" Test gewählt, weil er auf den reaktiven Sauerstoff-Spezies Peroxylradikal, Hydroxylradikal und Peroxynitrit basiert, die auch im Humanstoffwechsel von Bedeutung sind. Camu-Camu von zwei verschiedenen Herkünften zeigten fast identische antioxidative Kapazitäten gegenüber Peroxylradikalen und Peroxynitrit – die höchsten Werte im Vergleich zu anderen bis jetzt untersuchten Säften. Die inhibierende Wirkung beider Proben auf Hydroxylradikale war dagegen deutlich unterschiedlich. Die eine Probe zeigte eine Wirkung ähnlich der von Blaubeer- oder Apfelsaft, die andere war deutlich wirksamer. Untersuchungen zu den Ursachen dieser teilweise gleichen, teilweise unterschiedlichen Reaktivität wurden begonnen. Neben der Ascorbinsäure dürften verschiedene Flavonolglycoside, die mit Hilfe der HPLC-MS identifiziert werden konnten, ebenfalls einen Beitrag zur antioxidativen Kapazität liefern.

Kennwörter:
Myrciaria dubia, Camu-Camu, Vitamin C, TOSC-Test, Antioxidanzien, Polyphenole.

Introduction

The Amazon basin is the largest tropical forest area in the world with an extension of nearly six million km² with an unusually high biodiversity. Yet, up to now only a small fraction of plant species has been studied profoundly enough to come to reliable assertions on their potential for sustainable use.

The camu camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh], a shrub from the Myrtaceae family, is one of the promising Amazonian fruits, which has obtained increasing attention since the last years due to the extremely high content of ascorbic acid (1000-3000 mg/100 g). The species occurs naturally in areas of periodic flooding, such as low lands around river courses and lakes. The greatest concentration of natural populations and varieties is in the western (Peruvian) part of the Amazon basin [1]. It occurs also frequently in the north western part of the Brazilian Amazon; its distribution extends into Venezuela and Columbia [1-3]. A more detailed review on the natural habitat, the botanical characteristics, the actual knowledge about the fruit pulp composition, processing and sensory evaluation of camu camu containing food has been published recently [4].

From the extremely high content of ascorbic acid and from the occurrence of anthocyanins and carotenoids the pulp of camu camu should present a noteworthy

* *Eine Übersichtsarbeit über Camu-Camu ist auf den Seiten 376 ff zu lesen.*

antioxidant capacity. They are reactive oxygen species (ROS) scavengers, which can play an important role in the prevention of ageing processes or illnesses like cancer or cardiovascular disease [5]. The objective of this work was to examine the antioxidant properties of camu camu fruit pulp, because, up to now, no studies on that important fruit characteristic have been published.

Numerous methodologies for measuring antioxidant capacities have been developed; yet, their respective results are not comparable with each other because of different test conditions. Furthermore, frequently artificial radicals are used as reference ROS [6]. We decided in favour of the **T**otal **O**xidant **S**cavenging **C**apacity (TOSC) assay. It is a relatively new assay that is based upon naturally occurring test ROS. In the year 1998 that assay was introduced by *Winston et. al* [7] for studies on the oxidative stress of marine organisms.

For the first time three different ROS with an important potential to damage biological tissues are used within the same assay: peroxyl radicals, hydroxyl radicals and peroxynitrite. The degree of inhibition of the ethylene yielding reaction of KMBA (α-keto-γ-methiolbutyric acid) with the three ROS by antioxidants is monitored with gas chromatographic quantification of the ethylene accumulated in the head space. An advanced and automatic version of that method was developed by our own group [8].

Its main advantages are avoidance of the need for frequent manual gas chromatographic injections, increase of the short shelf life of test solutions and improvement of data evaluation. The application of this test to several standard compounds revealed that the reactivity of the antioxidants towards the three ROS is different, that different reaction types (retarding or fast acting) can be classified and that even prooxidants can be detected by that method. Results of the first application of that advanced method to European fruit and vegetable juices [9] as well as to exotic fruits like açaí [10] showed that the assay is suitable to characterise the antioxidant properties of the test juice samples in much more detail than it is possible with other established methods.

Material and methods

Camu camu I: Frozen camu camu pulps (harvested in 2005, dry matter 7.9 %) were obtained from the Agricultural Cooperative in Tomé Açú in Belém, Brazil.

Camu camu II: Freeze dried fruit pulp (harvested in 2006) was obtained from INPA (Instituto Nacional de Pesquisa da Amazonia) in Manaus, Brazil.

UHQ (Ultra High Quality) water prepared with an UHQ-II system (ELGA, Siershahn, Germany) was used for all solutions. Diethylenetriaminepentaacetic acid (DTPA), 3-morpholinosydnonimine hydrochloride (SIN-1), α-keto-γ-methiolbutyric acid (KMBA), were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 2,2'-Azobis(2-methylpropionamidine) dichloride (ABAP), ferric chloride hexahydrate, ethylenediaminetetraacetic acid (EDTA) were obtained from Acros Organics (Geel, Belgium). Ascorbic acid was obtained from *Kraemer* and *Martin* (Sankt Augustin, Germany).

Preparation of samples

27.42 g of the camu camu freeze dried sample II was reconstituted with UHQ water to a water content of 93.3 %. That moisture content was chosen according to the literature values [4].

The juices of both camu camu were diluted with UHQ water to at least five different dilutions for each of the three ROS assays to cover the respective range from a low to high antioxidant capacity as complete as possible.

TOSC assay

The TOSC assay is based on the ethylene yielding reaction between the ROS (peroxyl radical, hydroxyl radical and peroxynitrite) and α-keto-γ-methiolbutyric acid (KMBA). The antioxidant or TOSC value ranges from 0 to 100. Samples with no ROS scavenging capacity produce a TOSC value of 0 %, because they have the same area under the curve as the control reaction. Compounds that suppress the ethylene formation entirely gain a TOSC value of 100 %, and prooxidants obtain a negative TOSC value. Details of the assay conditions were published elsewhere [7, 8, 11]. The time course of the ethylene formation during one hour at 37 °C was analysed and the data evaluation was done according to *Lichtenthäler* and *Marx* [9].

Identification of individual phenolic compounds by HPLC-MS

Individual phenolic compounds were identified by ion trap multi stepmass spectrometric fragmentation after high performance liquid chromatographic separation and UV-Vis diode array detection with small improvements according to a method described previously in detail [12]. Reversed phase chromatography allows the separation of flavonolglycosides, and fragmentation of the quasimolecular ions in ion trap mass spectrometry has been proven to be a valuable tool in structure elucidation of polyphenols [13]. The identification of individual compounds was conducted by the comparison of the UV-spectra and mass spectrometricfragmentation patterns with reference spectra stored in a spectra library.

Results and discussion

Antioxidant capacity of camu camu by the TOSC assay

The antioxidant capacities of the camu camu juice in comparison with some other European and Brazilian juices as blueberry, orange, apple, and açaí [9, 10] against peroxyl radicals (Px), peroxynitrite (Pn) as well as hydroxyl radicals (Hy) are presented in the *Fig. 1 - 3*. The samples were analysed in at least five different dilutions (100 µL sample in 1000 µL test volume) to cover the TOSC ranges as complete as possible. Based on the experimental data, dilution factors were calculated that correspond to TOSC values of 20, 50, and 80 % (*Table 1*). A high dilution factor means a better antioxidant capacity of the sample.

For all analyzed samples, a nonlinear correlation between the sample concentration and the antioxidant capacities could be observed. The dose-response curves (*Fig. 1 - 3*) have the advantage to show a complete range of the antioxidant capacity spectrum of the samples. Almost all analyzed fruit juices are most effective against peroxyl radicals, less potent against peroxynitrite and fewest against hydroxyl radicals. This behaviour is explainable by the different ROS reactivities. The peroxyl radicals are the most stable ones with the least reactivity, with a half-life of several seconds [5]. By contrast, the hydroxyl radical is extremely reactive; it reacts with nearly all molecules present in the test solution matrix. Therefore, the relative efficiency of antioxidants against hydroxyl radicals is regularly lower.

Both camu camu juice samples displayed almost identical TOSC values against peroxyl radicals. Compared to all of the studied European and açaí juices the camu camu juice is by far the most effective in scavenging peroxyl radicals (*Figure 1*).

According to the classification of *Lichtenthäler* and *Marx* (in descending order: group of red juices; vitamin added juices; citrus juices and others) the camu camu juice outstands the group of the red juices; it represents a "new", a higher classification level [9].

As against peroxyl radicals, both camu camu samples also presented a higher antioxidant capacity against peroxynitrite in comparison with the other juices studied (*Figure 2*). It was much higher than that of blueberry juice that was considered, according to *Lichtenthäler* and *Marx* [9], until now the one with the best results.

As mentioned above, generally the antioxidant capacities of juices and standard compounds against hydroxyl radicals are lower and far less diverse in comparison with those against peroxyl and peroxyni-

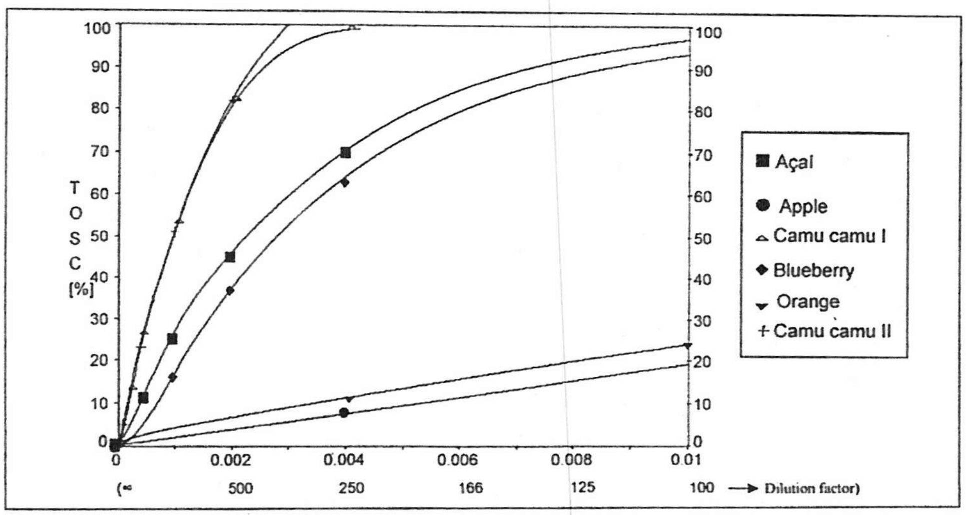

Fig. 1: TOSC against peroxyl radicals of camu camu juice in comparison with some other fruit juices

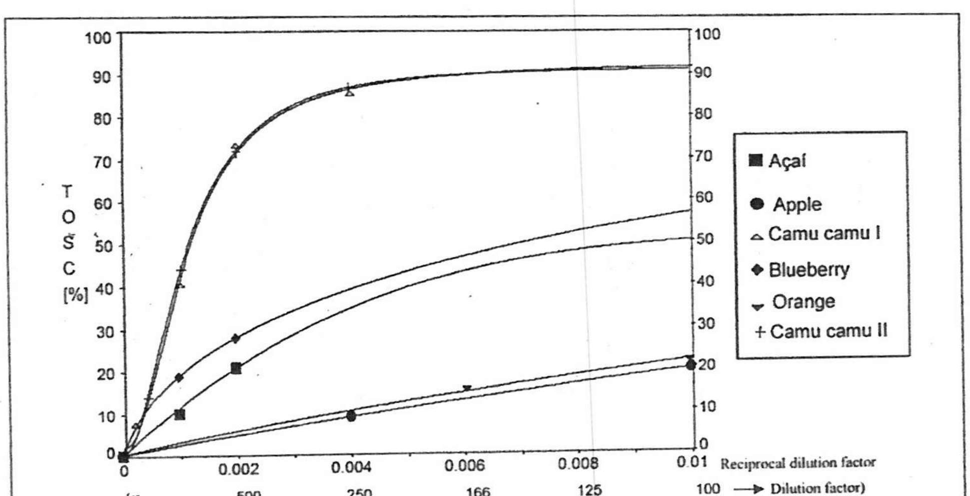

Fig. 2: TOSC against peroxynitrite of camu camu juice in comparison with some other fruit juices

trite. One of the camu camu samples (camu camu I) fits in; its TOSC values are very similar to that of blueberry juice. Indeed, the other (camu camu II) was notably more active (*Fig. 3*). That difference cannot be attributed to the ascorbic acid content because both samples present an average of 1500 mg/100 g. Furthermore, ascorbic acid is known to provide a very weak inhibitory effect against hydroxyl radicals, in some cases it shows an even prooxidative effect. Further studies are necessary to elucidate the partially equal and partially different reactivity of both camu camu samples. Polyphenolic compounds that are obviously partially responsible for the overall antioxidant capacity were identified by HPLC-MS as flavonolglycosides. Maybe, different contents of phenolic compounds are responsible for that phenomenon.

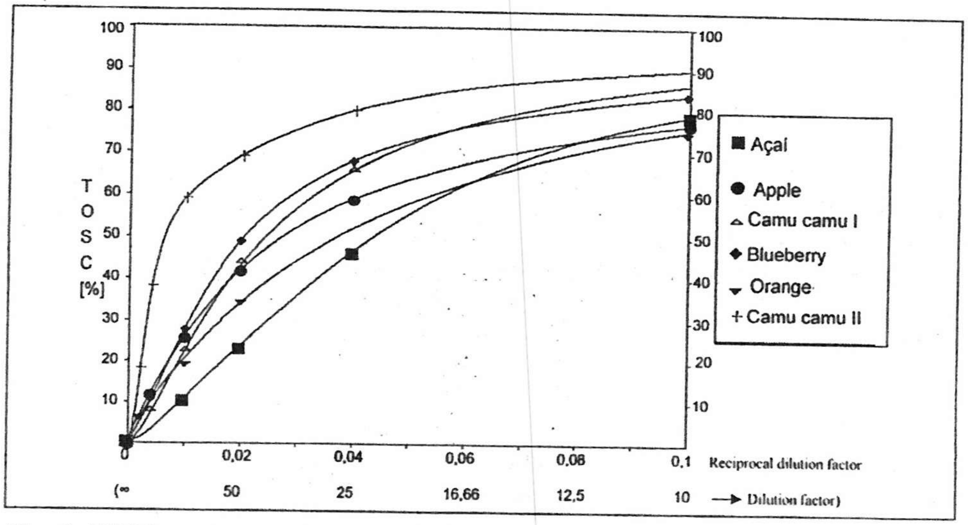

Fig. 3: TOSC against hydroxyl radicals of camu camu juice in comparison with some other fruit juices

Contributions of fruit compounds to the overall antioxidant capacity

To evaluate the contribution of ascorbic acid to the overall antioxidant capacity of camu camu against peroxyl radicals the juice (camu camu I) was diluted to achieve the same ascorbic acid concentration that was used for a standard ascorbic acid solution (1.66 mg/100 mL). The resulting TOSC of the camu camu juice was 68 % and that of the ascorbic acid standard was 49 %. The values indicate that ascorbic acid contributes to a major part to the total antioxidant capacity, as it could be expected against peroxyl radical, but other compounds or synergistic effects must be involved as well. The contributions of the camu camu ingredients to the overall antioxidant capacity against hydroxyl radicals are being studied.

Considering the fact that anthocyanin containing fruits like different berries and the palm fruit açaí show elevated antioxidant capacity, one of the compound classes to be considered is that of the anthocyanins. In camu camu fruits an overall content of anthocyanins of about 54 mg/100 g was reported [14]. However, only a moderate contribution of anthocyanins to the TOSC value is to be expected, because in comparison for example with the palm fruit açaí (up to approx. 450 mg anthocyanins/100 g) [10], its concentration is low. Therefore, we looked for another polyphenol group, the flavonolglycosides, which occurs frequently in fruit.

Identification of flavonolglycosides in camu camu fruit pulp

The combination of multi step mass spectrometric fragmentation with HPLC separation and UV-Vis diode array detection [12] allowed us to identify a number of flavonol-mono-glycosides with quercetin and myricetin, but not kaempferol, as aglycones and different carbohydrates attached. No hydroxybenzoic or hydroxycinnamic acids and no monomeric or oligomeric flavanols were detected in the samples. A characteristic HPLC chromatogram with the trace at 360 nm (absorption maximum for flavonolglycosides)

ROS	Hydroxyl radicals			Peroxynitrite			Peroxyl radicals		
TOSC values	20	50	80	20	50	80	20	50	80
Camu camu I	115	42	14	1725	829	366	3264	1092	524
Camu camu II	454	161	25	1969	864	382	2874	1027	522

Table 1: Calculated dilution factors of camu camu juice for TOSC against hydroxyl, peroxynitrite and peroxyl

is shown in *Fig. 4*. The three dominant compounds are a myricetin-pentoside, a myricetin-hexoside, and a quercetin-pentoside. Further investigations are in process that aim at the structural characterisation of the other flavonolglycosides present, the analysis of the type of attached sugars, and the quantification of these compounds as well as a correlation to their impact on the overall antioxidant capacity.

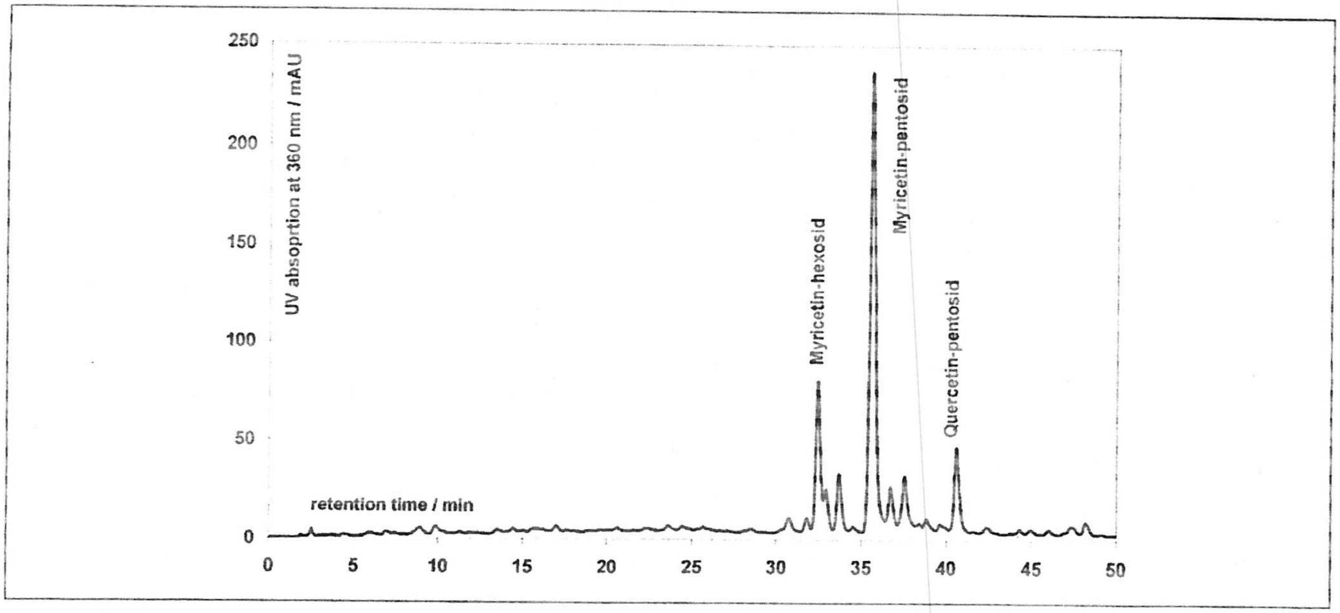

***Fig. 4:* HPLC chromatogram of camu camu juice extract at 360 nm.**

Conclusion

Camu camu fruit shows very high antioxidant capacity against the three ROS studied, higher than that of other foods that have been studied with that assay, up to now. Not only the extremely high content of ascorbic acid but also other compounds like anthocyanins or flavonolglycosides and/or synergistic effects (e.g. with citric acid) seem to contribute to the overall antioxidant capacity of camu camu fruit pulp. The camu camu is, until now, a hardly known fruit that presents a high potential to be explored as a functional food not only in the Amazon region but also in the big markets as Europe and USA. Further research needs to be done for a better understanding of the influence of the fruit constituents to the antioxidant capacity. Bioavailability studies should also be undertaken to evaluate its *in vivo* effectiveness.

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