Encapsulation, Properties, and Thermal Study of Red Biocolorant from Selected Plants Obtained Through Physical Extraction

by Yuyun Yuniati

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Encapsulation, Properties, and Thermal Study of Red Biocolorant from Selected Plants Obtained Through Physical Extraction

Renny Indrawati, Diah Mustika Lukitasari, Yuyun Yuniati, Heriyanto, and Leenawaty Limantara

Abstract—**The human perception on food is closely associated with its color. Since the standard manufacturing procedure often causes partial even total degradation of natural pigments, resulting in color fading, the addition of colorants becomes necessary. Natural colorant, produced from plants or animals, has health promoting effects, better safety, and need not any specific toxicity evaluation. However, the extraction method will be crucial in determining the properties of this biocolorant. In the present study, red biocolorant was prepared from selected local plants i.e., red spinach, red cabbage, beetroot, and dragon fruit, through physical extraction in order to avoid the using of organic solvents. Then, we applied the encapsulation technique and evaluated its coloring and antioxidant properties, as well as its stability against thermal treatment. The results showed that the encapsulated biocolorant of red spinach and beetroot exhibited red hue at pH range 2-11, whereas those of red cabbage and dragon fruit indicated color alteration at different pH. The prominent red hue intensity was found at pH 4 for encapsulated beetroot extract, which endured up to 10 days at aqueous buffered solution when stored in the dark at 20^{0}C. In addition, it underwent merely low degradation (~30%) during incubation at 60^{0}C for 30 minutes. The antioxidant activity of encapsulated biocolorant of beetroot was comparable to that of red cabbage, being higher than the others.**

Index Terms—**Biocolorant, coloring properties, encapsulation, red, thermal stability.**

I. Introduction

It is widely known that color is one of the prime factors in food choice, besides its physical appearance and odor. The appetite stimulators are red and yellow, while the most potential suppressor is blue [1]. Food industries have extensively used both synthetic and natural colorants in order to embellish their products, either giving new color or just improving the color after processing treatment that might cause fading. Although the properties of synthetic colorants are unrivaled, the health-aware consumers and regulatory authorities have unavoidably led the worldwide movement towards more natural colors in food [2]. The Royal Society of Chemistry recently published that over than ninety percent of European new products released during 2011 – 2016 have applied natural colors [3].

According to our latest market survey at several supermarkets in East Java, Indonesia, the use of non-synthetic colorant was dominant in baby food and (100%) and dairy products (60%), while its utilization on other categories was less than 20%, even none for instant meals. Our finding was in line with the common concept of human perception. The yellow beta-carotene (23%) and red carmine (21%) were predominantly employed beside the other natural sources such as annatto, curcumin, caramels, chlorophylls, and anthocyanin [4]. In fact, there is the 'carmine problem' which is related to its nauseating animal origin, aluminium content, microbiological issues, as well as its ability for inducing severe allergic reactions led to several public scandals [5]. Consequently, there is an urgent need for potential substitutes, coming from pigments or plant origin.

Some plants have been mentioned as the possible alternative for production of red biocolorant, i.e. dye sorghum (*Sorghum bicolor*), fruit of *Optunia stricta*, beetroot (*Beta vulgaris* L.), dragon fruit (*Hylocereus polyrhizus*), roselle (*Hibiscus sabdariffa*), and other plants of the Amaranthaceae [6]–[11]. Moreover, the subsequent concern is addressed to the preference of extraction and concentration method that should be able to compromise the instability of natural pigments, inexpensive, food-grade process, and environmental friendly. To the best of our knowledge, there is only a limited number of study in the production of red biocolorant which relied on solvent-free extraction and nonthermal processing.

In the present work, we encapsulated the red biocolorant, physically extracted from red spinach, red cabbage, beetroot, and dragon fruit, and then evaluate its properties and thermal stability. The encapsulation procedure is followed by lyophilization to give concentrated red biocolorant in powder form. Reconstitution of red biocolorant in buffered solution was intended to verify the influence of pH on its coloring properties and thermal stability. The antioxidant assay was carried out to examine the potency of these red biocolorants as functional food ingredients.

II. Materials and Methods

A. Materials

The red spinach (RS), red cabbage (RC), beetroot (BR), and

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Renny Indrawati, Yuyun Yuniati, and Heriyanto are with Ma Chung Research Center for Photosynthetic Pigments and Chemistry Study Program, Universitas Ma Chung, Malang, Indonesia (e-mail: renny.indrawati@machung.ac.id).

Diah Mustika Lukitasari is with Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Malang, Indonesia.

Leenawaty Limantara is with Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, and Universitas Pembangunan Jaya, Jakarta, Indonesia.

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dragon fruit (DF) were originated from locally grown plants sold at local grocery in Malang, East Java, Indonesia. Maltodextrin DE 10-12% (Yishui Dadi Corn Developing Co. Ltd., China) was used for encapsulating material. Solvents and reagents were commercially available from Merck Co. & Inc., USA and Sigma Aldrich Co., Germany. Chemicals for buffered solution were obtained from Lianyungang Chameleon Technology Co. Ltd., China (citric acid and sodium hydrogen phosphate) and Amresco Inc., USA (sodium azide).

B. Extraction and Encapsulation Procedure

All plants samples were rinsed with tap water, blotted dry, peeled (beetroot and dragon fruit), and cut. Then, physical extraction was performed by means of slow juicer HH-SBF11 (Hurom, USA) without any water addition. The collected extract was homogenized with Maltodextrin (5 % w/v), and kept frozen overnight prior to lyophilization for 48 hours at -49 ^{0}C under low pressure 0.04 MPa (Labconco-Freezone 2.5 L Benchtop, USA). The dried encapsulated extract (moisture content below 10 %) was ground to give red biocolorant in powder form, stored at freezer (-18 ^{0}C) until it was used for further analysis.

C. Preparation of McIlvaine Buffer

The McIlvaine buffer was prepared according to [12] with slight modification. Citric acid (less than 2 % w/v), sodium hydrogen phosphate (less than 2 % w/v), and sodium azide (0.02% w/v) were dissolved in distilled water to create a series of buffered solutions at pH 2 – 11.

D. Tinctorial Strength and Stability Evaluation

The encapsulated red biocolorant (0.1 % w/v) was redissolved in McIlvaine buffer in order to evaluate the coloring capacity, measured as tinctorial strength = [maximum absorbance × 100]/sample weight. The biocolorant in buffered solutions were stored in Climate Chamber ICH110 (Memmert, Germany) at 20 ^{0}C with 15 % relative humidity for certain period of time under darkness. Thermal study was carried out at 60 ^{0}C for 30 minutes using waterbath in duplicate samples.

Spectroscopy measurement was accomplished by means of Spectrophotometer UV-1800 (Shimadzu, Japan), while the color measurement was performed using Colorflex EZ No.45/0 (HunterLab, USA). Hue was calculated as the angle having tangent b*/a*. Change of colors was calculated as ΔE from the Hunter L*, a*, and b* values using determined equation [13].

E. Antioxidant Assays

Antioxidant activity and IC value of encapsulated extract was determined against DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical [14]. The encapsulated biocolorant was first dissolved in water at 1000 ppm, then a series of dilution was prepared using methanol, followed with addition of 0.2 µM DPPH solution. Samples were incubated for 30 minutes in the dark at room temperature, and the remaining DPPH radicals was quantified using absorption set at 517 nm. The IC_{50} value represents the weight of sample required to scavenge 50 % of the available DPPH radicals.

III. Results and Discussion

A. Encapsulated Red Biocolorant

Red biocolorant from plant origin could be empowered due to the presence of some natural red pigments, i.e. lycopene, anthocyanins, betalains, and betacyanins. Compared to other pigment groups with different color appearance, most of red pigments have high polarity and exhibit favorable solubility in water. Since the main constituent of some parts of the plants is water, those pigments may be concurrently squeezed out from the intact structure via free and bound water. The red spinach, red cabbage, beetroot, and dragon fruit were chosen and subjected to physical extraction by means of slow juicing instrument, which is applicable to parts of plants that do not have rigid structure and contain enough water. Furthermore, the extract was encapsulated and lyophilized in order to reduce the water content and hence increase its shelf life.

In the present work, four types of powdered biocolorant were generated from the selected raw materials. The red spinach and beetroot gave vivid red powder, while red cabbage and dragon fruit provided purplish red powder. The particle attribute was investigated by using Scanning Electron Microscopy (SEM), revealing the typical irregular shape of freeze dried material with average particle dimensions ranging from 54.61 ± 9.50 µm × 85.80 ± 10.92 µm. Since the microcapsules may range from 0.2 – 5000µm in size [15], our encapsulated biocolorant could be attempted as microencapsulation of plant pigments. As detailed by [16], one reason for using microencapsulation technologies is to facilitate the heat- and light-labile ingredients like many pigments, so that their shelf life and release time could be tuned. Despite the fact that spray drying may produce more homogenous form of microcapsules, the high temperature of inlet promoted partial change of the heat-labile pigments which led to faster degradation [17], [18]. Freeze drying (lyophilization) was chosen as nonthermal processing which indicated lower degradation.

Fig. 1. Color disparities of encapsulated red biocolorant prepared from red spinach (a), beetroot (b), red cabbage (c), and dragon fruit (d), redissolved in buffered aqueous solution at pH 2 – 11 and after storage (20^{0}C, dark).

B. Coloring and Antioxidant Properties

Fig. 1 shows the color disparities of encapsulated

biocolorant in McIlvaine buffer at various pH. The hue and color intensity were exceptionally influenced by pH value as well as pigments composition of each raw materials.

The extract of red spinach and beetroot gave appealing red color in buffered solution at wide pH range, yet the intensity was slightly decreased on very acidic and basic condition. The extract of dragon fruit showed almost comparable intensity shift with purplish hue. On the other hand, the color of red cabbage extract was interestingly varied depends on the pH value, having the highest intensity of bluish hue in basic environment. Quantitative measurement for this finding was depicted as tinctorial values in Fig. 2. The encapsulated biocolorant of red spinach, beetroot, and dragon fruit denoted a polynomial relationship between pH and its tinctorial strength with the maximum at slightly acid to neutral region (pH 4–8), whereas that of red cabbage was fairly different, having the tinctorial maxima at pH 2 (red) and about pH 9–10 (blue). Overall, encapsulated red biocolorant from beetroot exhibited superior tinctorial strength, meaning that the stronger coloration could be released by the equal dyes concentration.

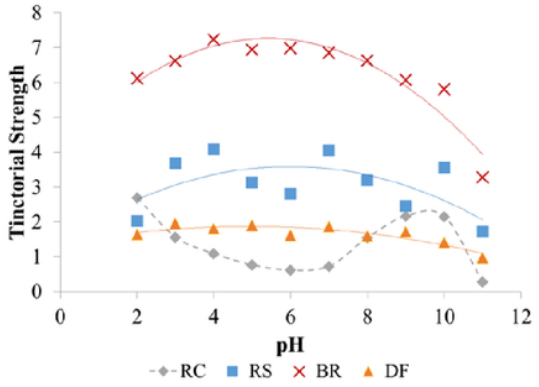

Fig. 2. Tinctorial strength of encapsulated red biocolorant prepared from red spinach (RS), beetroot (BR), red cabbage (RC), and dragon fruit (DF) at concentration of 0.1 % (w/v) in buffered aqueous solution at pH 2—11.

The red spinach, beetroot, and red dragon fruit are known to have dominancy of betalains on its stems, leaves, and roots. It consists of red-violet betacyanins and yellow betaxanthins groups, which absorb light near the maxima at 535 nm and 480 nm, respectively [19]–[21]. The diversity in composition and content of pigment fractions on both groups, as determined by genetic information, steps of growth/maturity, as well as environmental factors, will result in color variety. Instead of betalains, the anthocyanins group dominates in the biocolorant extract from red cabbage [22]. In accordance with the characteristic of anthocyanins, cabbage extract had red color at acidic solution, almost transparent at neutral, and turned to blue and even green at alkaline solution. Although all red biocolorant of the present work may exhibit similar color in their powder form, each revealed distinct coloring properties.

It is also interesting to know that most natural pigments also play a biological role as antioxidant. Table I listed the IC_{50} value of encapsulated red biocolorant, in which a lower IC_{50} indicates greater antioxidant activity. The biocolorant of red spinach and beetroot obtained through physical extraction possessed higher antioxidant activity than those of red cabbage and dragon fruit, gaining more preeminence besides their excellent red color and strong coloration.

TABLE I: IC_{50} VALUES OF ENCAPSULATED RED BIOCOLORANT ON DPPH-RADICAL SCAVENGING ACTIVITY

Origin of Encapsulated Red Biocolorant	$IC_{50}^{*} \pm$ SE (ppm)
Red Spinach (RS)	1558.00 ± 22.29
Beetroot (BR)	1921.88 ± 1.76
Red Cabbage (RC)	4560.23 ± 266.98
Dragon Fruit (DF)	5299.99 ± 1521.25

*Values obtained from regression lines with a good coefficient of determination ($r^2 \geq 0.85$), SE standard error.

Moreover, the stability of these biocolorant was monitored during certain storage period at ambient temperature. The fluctuation of hue in pH 4 to 8 became our focus since most samples had stronger coloration, as well as food and drugs environment also generally cover this range. Fig. 3 revealed that the change of hue was significantly affected by pH and time of storage. The acidic solution at pH 4 was the most favorable condition for all biocolorant, causing mild change of hue during storage. The hue of red spinach and dragon fruit extract tend to increase as time changes, whereas that of red cabbage extract was found to decrease at pH 6–8. A distinctive characteristic was found at beetroot extract, which showed superior stability at pH 4 (10 days), 7 (8 days), and 8 (8 days), but brought out poor stability at pH 5 (2 days) and 6 (4 days). The color of encapsulated beetroot extract was easily changed to brownish yellow at pH 5 and 6, unlike the consistent red hue in the other pH values. The increase of lightness (L*) value was detected in all samples, revealing the occurrence of pigments degradation and naturally color fading.

Previous study of the red spinach done by [23] had emphasized the stability of anthocyanin fraction from its fruit, which was more stable at pH 5 and 6 rather than at pH 4. However, the dominance of anthocyanin groups might not be appeared in the encapsulated red biocolorant from red spinach. According to [24], the stability of betanine fraction from beetroot had great stability at pH 3.5, even also signifying greater antiradical activity which not easily degraded under illumination treatment, being in line with our detection. In addition, it was supposed that the natural acidic environment of dragon fruit (pH 5) should be most favorable to retard degradation of its pigments. In fact, the study of [9] revealed that the color of dragon fruit juice could be maintained up to 80% after 3 weeks dark storage at 4^0C in pH 3. Our findings complemented the foregoing research that mostly employed solvent-based extraction, so that might not necessarily be applied directly in the food and pharmaceutical industries. Yet the stability evaluation of isolated pigment would be a worthwhile basis to explain our data.

C. Thermal Study

The thermal study was aimed to estimate the effect of middle heating treatment to the color change of encapsulated

red biocolorant in buffered solution. This information could be the reference to avoid significant color adjustment after thermal processing. We observed the color change as ΔE value which consider the shift of lightness (L*), redness (a*), as well as yellowness (c*) value. Thus, smaller value of color difference (ΔE) manifested preferable stability.

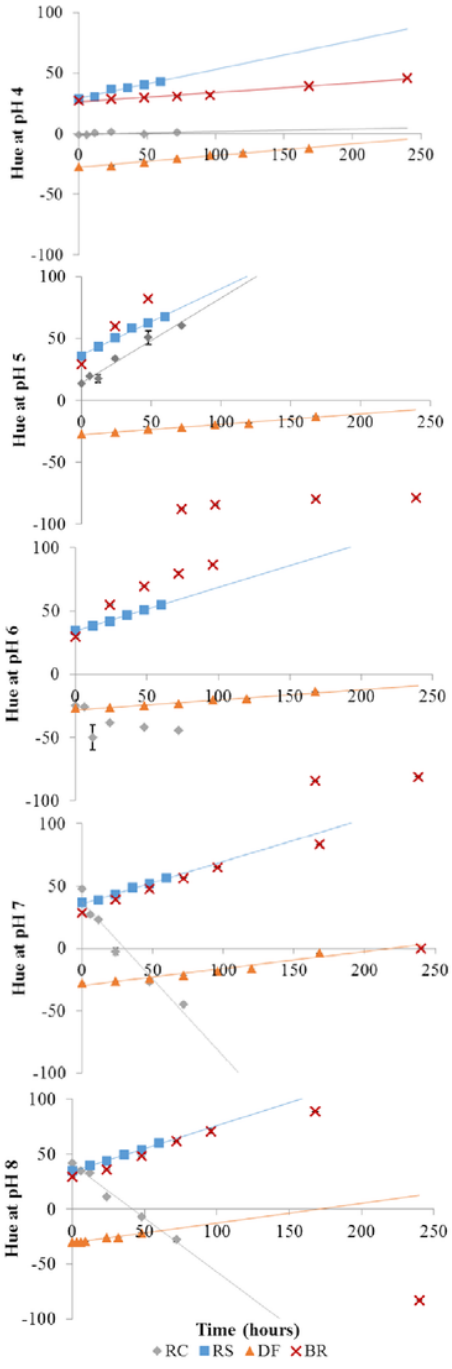

Fig. 3. The hue evolution of encapsulated red biocolorant prepared from red spinach (RS), beetroot (BR), red cabbage (RC), and dragon fruit (DF) in buffered aqueous solution at pH 4 – 8 within dark storage in 20^{0}C.

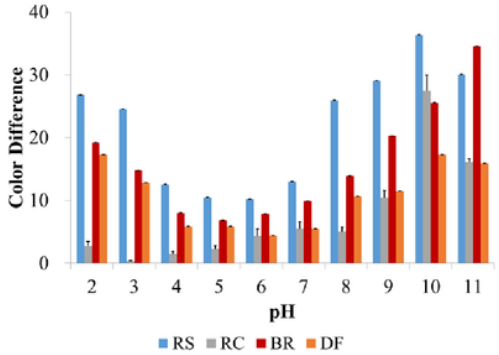

Fig. 4. Color difference (ΔE) of encapsulated red biocolorant prepared from red spinach (RS), beetroot (BR), red cabbage (RC), and dragon fruit (DF) in buffered aqueous solution at pH 2–11, after thermal treatment at 60^{0}C for 30 minutes.

The thermal study gave a consistent result concerning pH-dependent stability of these encapsulated red biocolorant. Fig. 4 yet again revealed that most red biocolorant on the present work exhibited distinguished stability at pH range 4 to 8. Although the extract of red spinach showed vivid red color when reconstituted, it experienced greatest discoloration after incubated for 30 minutes at 60^{0}C. The extract of beetroot underwent a slightly smaller color change than that of red spinach. Although the red powder produced from dragon fruit and red cabbage had lesser color difference, both did not consistently displayed red hue. The extract of dragon fruit could be a candidate for purple colorant, whereas that of red cabbage is rather fitted as blue colorant with an alkaline treatment.

D. Future Prospect and Outlook

In the point of view of chemistry, indeed there is nothing more stable than the synthetic dye. Nevertheless, the use of biocolorant has several advantages i.e., it is inescapable safe colorant for both the body and environment, exploited from renewable sources and hence supporting appropriate use of local wisdom, could be extracted without bearing any toxic chemical waste, and possesses health benefit upon its consumption. Additionally, the instability of biocolorant could be useful as bio-indicator, such as detection of food spoilage that causes pH alteration, adequacy of food processing or heat treatment, as well as biosensor of shelf life detection in intelligent packaging system. In case the minimal discoloration is needed, incorporation of biocolorant from several raw materials could be an option. For instance, the extract of red spinach may correct the instability of beetroot extract during prolonged storage at pH 5 and 6, and then a little amount of dragon fruit extract could support its color within heating treatment. Elucidation of most stable pigment fraction among those materials is also being the part of our current project.

IV. Conclusion

Four types of vivid red powder were effectively produced through simple physical extraction and lyophilization step

from red spinach, beetroot, red cabbage, and dragon fruit. Considering the coloring capacity, hue, and antioxidant activity, red spinach and beetroot met the superior candidate of eco-friendly red biocolorant. The beetroot extract showed good stability during dark storage up to 10 days in 20^0C, especially at pH 4, 7, and 8. The extract of red spinach and dragon fruit could be incorporated in order to accomplish its discoloration at pH 5 and 6 as well as in thermal processing.

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Renny Indrawati was born in Malang, East Java, Indonesia, on May 29, 1986. She received the undergraduate degree (bachelor in food technology) with a cum laude honor from Brawijaya University, Indonesia, in 2008. She began her career in research by joining Ma Chung Research Center for Photosynthetic Pigments (MRCPP) as a research assistant in 2009, and she is deeply interested in the development of functional food related to natural pigments. In 2011, she graduated with a summa cum laude honor from Satya Wacana Christian University, Indonesia, majored in Chlorophyll and Carotenoid. She subsequently finished the second master degree by research (master of international natural science) in 2012 at Kwansei Gakuin University, Japan.

Currently, she is a researcher at MRCPP as well as a lecturer assistant at Chemistry Study Program, Universitas Ma Chung, Indonesia. She has been involved in some prestigious national research grants, such as Excellent Research on National Strategy, Competitive Research Grant, and National Innovation System Research Grant. Her current research interest lies in the fabrication and stability studies of functional food colorants from natural resources.

Ms. Indrawati is an author and co-author of Indonesian peer-reviewed papers as well as several international publications. She has been awarded for a double degree scholarship by Indonesian Government to pursuit her graduate study for two years and a half. She received the award as one of the best presenters in Natural Pigments Conference for South-East Asia in three consecutive years.

Diah Mustika Lukitasari was born in Malang, Indonesia, in 1990. She received bachelor and master degree in science and food technology from the Brawijaya University, in 2012 and 2015, respectively. Her major field of study is functional food and dietary supplement.

In 2012, she was a practical work assistant for manual laboratories of work and general microbiology in the Department of Agricultural Technology, Brawijaya University, Indonesia. In 2016, she joined Ma Chung Research Center for Photosynthetic Pigments (MRCPP), Universitas Ma Chung, Indonesia, as a research assistant. She has experienced in several research field, including the development of functional food, immunology of dietary supplements, microencapsulation of pigment, food colourants, and antioxidant activity of natural compounds.

Yuyun Yuniati received her doctoral degree in chemical engineering from Institute of Technology Sepuluh Nopember Surabaya, Indonesia, in 2013. Currently, she is a head of Chemistry Study Program at Universitas Ma Chung, Indonesia. She is a member research fellow in Ma Chung Research Center for Photosynthetic Pigments (MRCPP). She has focused her research in the field of functional food development, processing technology, kinetic and catalysis, as well as industrial chemistry.

Heriyanto received his B.Sc in Chemistry from Satya Wacana Christian University, Indonesia, in 2004. In 2008 and 2009, He obtained M.Sc as a double degree program in Biology from Satya Wacana Christian University, Indonesia and in Chemistry from Kwansei Gakuin University, Japan. Currently, he is a Ph.D student in the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Poland.

He worked as a research assistant from 2004 to 2009 and then continues his work as a researcher at Ma Chung Research Center for Photosynthetic Pigments (MRCPP), Universitas Ma Chung, Indonesia. Since 2015, he has been invited as a lecturer in the Department of Chemistry, Universitas Ma Chung, Indonesia. He has an experience in the analysis of natural pigments for almost 14 years. His research interests include pigment analysis, analytical chemistry, spectroscopy and chromatography, as well as biochemistry of photosynthetic pigments.

Leenawaty Limantara finished her graduate study in Kwansei Gakuin University, Japan, majored in the physical chemistry related to photosyntetic pigments in light harvesting antenna. She has been involved in several post-doctoral research in Japan, United States, United Kingdom, and Germany.

She a invited lecturer and principal investigator in Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung. She has been engaged in many studies related to chlorophyll since 1991 and received many research grant from both national and international institution. Currently, she is the rector of Universitas Pembangunan Jaya, Jakarta, Indonesia.

Dr. Limantara has published her work in many international journals, chapter of book, review, and national peer-reviewed journal. She is the founder as well as chairwoman of the Association of Pigment Researchers in Indonesia. She is also the first Indonesian Ambassador Scientist for the Alexander von Humboldt Foundation, Germany.

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