Biochar-supported Cu nanocluster as an electrochemical ultrasensitive interface for ractopamine sensing

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Article Info

Abstract

Electrochemical sensors actually involve an electrocatalytic process involving an efficient and selective energy conversion that is related to the morphology and size of the interface of the modified materials. Ultrasmall nanoclusters or single atoms generate a greater catalytic ability than normal nanomaterials. In this study biochar-supported Cu nanoclusters (CuNCs@CNFs) were fabricated via a carbon confinement synthesis method toward ultrasensitive electrochemical sensing of ractopamine (RAC). RAC is a β-adrenergic receptor agonist that is illegally used as a feed additive to significantly improve muscle accretion, resulting in RAC accumulation in meat-based food products. The unique structure of CuNCs@CNFs and the interconnectivity between the CuNCs and the CNFs enable the nanocomposite to significantly enhance conductivity and electrocatalytic activity. Using the CuNCs@CNFs-based sensor, RAC was determined with a high sensitivity of 1641 μA μM⁻¹ cm⁻². The feasibility of detecting RAC in spiked meat samples was also carried out with satisfactory recoveries ranging from 91.39 % to 94.58 %.

1. Introduction

Ractopamine (RAC) is classified as a β-adrenergic receptor agonist. RAC was originally developed for the treatment of pulmonary disease and asthma. However, today it is illegally used in animal feed to enhance muscle content and decrease adipose tissue deposition in order to increase muscle growth rate (Armstrong et al., 2004). Mounting evidence has demonstrated that excessive intake of RAC causes adverse health effects such as acute poisoning, nervousness, and cancers (He et al., 2011; Rajkumar et al., 2013; Liu et al., 2012). To date, RAC has been banned or restricted in 160 countries, including China, members of the European Union (EU) and Russia. While in some countries RAC is widely used, it is estimated that 60 %-80 % of pigs raised are fed the RAC (Aroeira et al., 2021). Due to the high risk of RAC, the Codex Alimentarius Commission (CAC) adopted the first-ever maximum residue levels (MRL) for RAC hydrochloride as 10 μg kg⁻¹ for beef and pork in 2012. The U.S. Environmental Protection Agency (EPA) has adopted a 2.5 mg L⁻¹ maximum RAC contaminant level for meat, and the World Health Organization (WHO) standard for the maximum permissible levels of RAC in meats is 5 mg kg⁻¹ (Bai et al., 2014). China and the EU do not tolerate residues of the RAC in meat. Nevertheless, the illegal addition of RAC has been consistently reported (Zhang et al., 2022; Liu et al., 2022a). For example, the Rapid Alert System for Food and Feed (RASFF) of the EU reported RAC residue in beef livers in 2012. Chinese custom detected RAC residue in imported beef and pork in 2017 and 2019. Therefore, the ultrasensitive monitoring of RAC in food is of significant importance for food safety and human health.

To date, various methods for RAC detection have been established (Simon et al., 2018; Han et al., 2021; Muthumariyappan et al., 2019), among which electrochemical methods are advantageous for their ease of operation, rapid response, high sensitivity, and suitability for rapid sensitive detection and spatial–temporal analysis (Poo-arpong et al., 2019; Shen et al., 2022). Until now, different electrochemical methods have been reported for the monitoring of RAC in food samples (Liu et al., 2012; Muthumariyappan et al., 2019).

However, the sensitive electrochemical detection of RAC in food is still challenging, due to their high overpotential as well as poor response signals of RAC on ordinary electrode surfaces (Wu et al., 2012). Modifying nanomaterials with high electrocatalytic activity on the surface of electrodes is regarded as the most effective way to enhance the sensing performance of electrochemical sensors. Nanomaterials such as metal or metal oxide nanoparticles (Bai et al., 2022; Wang et al., 2020),
metal–organic framework (MOF) (Yue et al., 2022), carbon nanomaterials (Vatandost et al., 2020), etc. with large surface areas, numerous electrochemical active sites, and fast electron-transfer kinetics are the most promising candidates. Among them, transition metal nanomaterials such as CuO (Sudha et al., 2018), FePO$_4$ (Wang et al., 2018), Mn$_3$(PO$_4$)$_2$ (Zhang et al., 2015), ZrO$_2$ (Rajkumar et al., 2013) and Co$_3$O$_4$ (Xia et al., 2014) have been used for the sensitive electrochemical detection of RAC due to their abundant reserves, low manufacturing cost, and high catalytic ability. Moreover, electrochemical sensors utilize an electrocatalytic process needed for the efficient and selective energy conversion which is primarily related to the morphologies and size of the interface of the modified materials (Zhang et al., 2019). Ultrasmall nanoclusters or single atoms (SAs) generally generate greater catalytic ability than normal nanomaterials because of high atom utilization and prominent size effects (Singh et al., 2021; Wang et al., 2017). Yet the fabrication of SAs faces significant challenges since the SAs cluster easily under real working conditions (Singh et al., 2021). Studies have proven that porous carbon nanofibers (CNFs) can produce space confinement effects during the in situ carbothermic reduction of metal ions, prevent the gathering of nanoparticles and further increase of nanoparticles size, forming tiny nanoclusters, as well as endowing the electrode materials with numerous defects, large surface areas, high electrical conductivity, and many mass/ion transfer channels (Liu et al., 2019; Kang et al., 2022; Xiao et al., 2022). CNFs are, therefore, ideal supporters for the synthesis of nanoclusters needed in order to develop electrochemical sensor platforms with high performance.

Herein, we report the fabrication of an ultrasensitive and selective detection method of RAC in food using a novel biochar-supported Cu nanocluster (CuNCs@CNFs) constructed via a carbon confinement synthesis method. Porous CNFs in this work provide a space confinement effect during the in situ carbothermic reduction of Cu$^{2+}$, resulting in tiny CuNCs, which act as supporters and reductants of CuNCs. In the sensing of RAC, the synergistic effects of CNFs with superior conductivity and mesopore structure, the CuNCs with high electrocatalytic activity, and the interconnectivity between CuNCs and CNFs allowed for the high sensing performance of the CuNCs@CNFs. A linear response from 2 to 850 nM with a lower limit of detection (LOD) of 0.05 nM, and an ultrahigh sensitivity of 1641 $\mu$A$\cdot$M$^{-1}$ cm$^{-2}$ were experimentally observed using CuNCs@CNFs-based sensors. The feasibility of detecting RAC in different kinds of meat was successfully carried out with satisfactory recoveries ranging from 91.39 % to 94.58 %, which is comparable to that obtained using HPLC methods.

Material and methods

2.1. Chemicals and reagents

Cupric chloride dihydrate (CuCl$_2$-2H$_2$O, 99.99 %), dopamine (DA, 98 %), Glucose (Glu, 99.5 %), uric acid (UA, 99 %), clenbuterol (Cle, 1.0 mg/mL), fenoterol (Fen, 99 %), salbutamol (Sal, 95 %),

![Scheme 1. The synthesis process of CuNCs@CNFs nanocomposite and electrochemical detection of RAC based on CuNCs@CNFs/GCE.](image-url)
phenylethanolamine A (Phe, 98 %) and ascorbic acid (AA, 99 %) were purchased from Sigma-Aldrich, racoptamine (RAC) was purchased from Dr. Ehrenstorfer GmbH and used as received without any additional purification. All other reagents are of analytical grade and were purchased from Tianjin Tianli Chemical Reagent Co., Ltd. (Tianjin, China). All solutions were prepared using ultrapure water (18.2 MΩ cm) and stored at 4 °C, deaerating with high-purity nitrogen before experiments. Bacterial cellulose (BC) was obtained by inoculating tofu yellow slurry water with *Acetobacter xylinum* followed by 5 day’s culture (Tabuchi et al., 1998).

### 2.2. Synthesis of CuNCs@CNFs

The synthesis process of CuNCs@CNFs is shown in Scheme 1. Firstly, BC was decolorized with 0.1 M NaOH and washed with ultrapure water to neutral. Then, BC was soaked in 0.001 M CuCl₂·2H₂O for 12 h to acquire BC-CuCl₂ by electrostatic adsorption of hydroxyl or carboxylic acid groups on BC to CuO₃ [2]. After stirring continuously for 12 h, the BC-CuCl₂ was washed 5 times with ultrapure water to remove excess CuCl₂. Then the BC-CuCl₂ was dried in a vacuum freeze-drying equipment for 3 h. Finally, CuNCs@CNFs were obtained by carbonizing BC-CuCl₂ at 900 °C for 1 h in nitrogen flow. The temperatures were started from room temperature to rise to 2°C min⁻¹ to 180°C, then at 1°C min⁻¹ to 500°C and at 2°C min⁻¹ to 900°C, followed by cooling naturally to room temperature in nitrogen flow.

### 2.3. Apparatus

All electrochemical experiments were tested on a CHI660D electrochemical workstation (Chenhua, Shanghai, China). During all the tests, a platinum wire electrode was used as the counter electrode, an Ag/AgCl electrode was used as the reference electrode and a glassy carbon electrode (GCE, Φ = 3 mm) was used as the working electrode. Morphology of the as-prepared nanocomposites was investigated with field emission scanning electron microscope (FESEM, SU-8020, Hitachi, Japan) and transmission electron microscope (TEM, JEM-2800, Jeol, Japan). The crystallographic structure was carried out by X-ray diffraction (XRD, Smart Lab, Rigaku) with Cu Kα radiation in the 2θ range of 10°–90°. X-ray photoelectron spectroscopy (XPS) was performed by ESCALAB 250xi (Thermo Fisher Scientific, America). Energy-dispersive spectroscopy (EDS) was performed by X-Max 80/EX-270 (HORIBA, Japan).

### 2.4. Preparation of nanomaterials modified electrodes

The synthesized nanomaterials, CuNCs@CNFs and CNFs, were respectively dispersed in ethanol and ultrapure water (v/v = 1:1) to form a 3.0 mg/mL suspension. Subsequently, 5 μL of the above dispersed solution were respectively modified on the GCE surface and dried in air under room temperature. Before measurement, the used buffer solution, 0.01 M phosphate buffer saline (PBS, pH = 7.4), was purged with nitrogen for 10 min to remove dissolved oxygen, and the CuNCs@CNFs/GCE or CNFs/GCE were scanned in blank PBS buffer solution between 0.1 and 0.9 V at 50 mV/s until the cyclic voltammogram (CV) sweeps being steady to activate the electrodes.

### 2.5. Sample preparation

Meat purchased from market were used as detection samples. The preparation processes were carried out according to previous work (Wu et al., 2012; Koerthi et al., 2022). 2 g of meat sample was first crushed, 4 mL 0.1 M HClO₄ was then added to the crushed sample followed by ultrasonication for 30 min. The samples were kept in water at 80 °C for 30 min. Next, the supernatant of the above mixture was collected by centrifuging at 6000 rpm (4724 rcf) for 10 min. The obtained supernatants were extracted using 5 mL of ethylacetate after adjusting their pH to 10 with 0.01 M NaOH, then RAC was back-extracted into HCl solution. The extraction and extraction were repeated several times. Finally, the RAC solutions were diluted to 10 mL with PBS (0.01 M, pH 7.4).

### 3. Results and discussion

#### 3.1. Characterization of CuNCs@CNFs

The morphology and microstructure of BC and the CuNCs@CNFs were visualized using FESEM (Fig. 1a, b). It revealed that the fiber network structure of BC (Fig. 1a) remained unchanged after the pyrolysis process except the surface became rougher (Fig. 1b). In Fig. 1c, numerous light spots that were dispersed in the TEM image of CuNCs@CNFs and labeled by red circles. These dispersed light spots are considered to be CuNCs with diameters of 1.25–2.75 nm (Fig. 1e). EDS imaging (Fig. 1d) revealed the co-existence of C, Cu, and N within the CuNCs@CNFs nanocomposite materials. The existence of C and Cu agrees with the elemental compositions of the CuNCs@CNFs, while the N element may have derived from the pristine BC that were cultured within the N-rich tofu yellow slurry water. The elemental composition of the CuNCs@CNFs is then investigated and characterized using XPS. The images reveal a wide survey spectra of the CuNCs@CNFs (Fig. 1f), showing that C and Cu co-exist on the surface of the CuNCs@CNFs. In the high-resolution Cu 2p spectrum, two main peaks with binding energies at 952.3 and 932.4 eV correspond to Cu 2p₁/₂ and Cu 2p₃/₂, respectively (Fig. 1h). The above characteristics confirmed the successful carbon confinement synthesis of CuNCs on the surface of the CNFs (Mahmoud and Hattam, 2022).

Furthermore, the crystal structure of the CuNCs@CNFs was investigated using the XRD spectrum (Fig. 1i). All diffraction peaks corresponding to the (1 1 1), (2 0 0), and (3 1 1) planes of Cu (JCPDS no. 70–7038) (Liu et al., 2022a), except the broad diffraction peak of carbon at approximately 25°. The intense and sharp peaks of Cu suggested that the sample is of high purity and excellent crystallinity and agrees with the high-resolution TEM images (HRTEM) (inset of Fig. 1c). The pore size distribution of the CuNCs@CNFs was also tested using the nitrogen adsorption-desorption isotherms (Fig. S1). As shown in Fig. 1g, a certain number of mesoporous (2–50 nm) were present in CuNCs@CNFs, enabling the CuNCs@CNFs with an enlarged surface area and numerous channels for mass transport. The pyrolysis process endowed the carbon supports with porous structure, and also yields a thermal reduction environment for the synthesis of the CuNCs. More importantly, the mesopores within the CNFs effectively prevents the increase in size of the CuNCs due to providing a space-limiting environment.

#### 3.2. Electrochemical properties of the CuNCs@CNFs

The electrocatalytic performance of different materials used for RAC sensing was studied via CV curves measured in 0.01 M PBS (pH 7.4). Fig. S2 shows the CV signals of the CuNCs@CNFs/GCE sensor with and without 200 nM RAC. No visible signal peak appeared in the blank PBS; however, a very strong oxidation peak was observed at 0.56 V in the presence of RAC, indicating a relatively high electrocatalytic activity of the CuNCs@CNFs/GCE sensor for RAC oxidation. For comparison, CVs of different electrodes were measured in 0.01 M PBS containing 400 nM RAC (Fig. 2a). CuNCs@CNFs exhibited the highest peak current and lowest peak potential than other materials, while bare GCE generated the lowest current signal and highest peak potential. For an electro-oxidation reaction, a higher peak current symbols a larger surface reaction area. A more negative half-wave potential means a higher electrocatalytic activity (Zhang et al., 2021). Therefore, the CuNCs@CNFs were considered to have the highest electrocatalytic activity and largest active surface area with respect to other nanomaterials. This can be attributed to the strong catalytic activity of Cu as well as the unique structure of the extra-small CuNCs located on the surface of the porous CNFs; thus, exposing significantly more Cu
active sites.

In order to further verify the above results, a double layered capacitance (C<sub>dl</sub>) that is proportional to the active surface area of the nanomaterial’s modified electrodes was determined (Zhang et al., 2019). CVs of the CuNCs@CNFs/GCE, CNFs/GCE, and bare GCE were tested at various scan rates (10, 20, 30, 50, 60, 80, and 90 mV s<sup>-1</sup>) in a potential window of 0.15–0.25 V where no apparent Faradaic processes occur (Fig. S3). Plots of the double layer currents at 0.2 V as a function of the scan rates of the three electrodes were illustrated in Fig. 2b, in which the slopes corresponding to their C<sub>dl</sub> are 74.08, 14.73, and 5.51 µF for the CuNCs@CNFs, CNFs, and bare GCE, respectively. This again verified that the CuNCs@CNFs had the largest electrochemically active surface.

The electrochemical sensing process of RAC using a CuNCs@CNFs/GCE sensor was explored by measuring the CVs at different scan rates ranging from 10 mV/s to 100 mV/s in the presence of 200 nM RAC (Fig. 2c). It can be seen that the anodic peak current of RAC increases progressively with an increase in scan rate. Whether the electrochemical reaction of RAC is a diffusion or a surface-controlled process can be determined by the following equations:

\[
i = a v^b
\]

\[
\log i = b \times \log v + \log a
\]

where \(i\) and \(v\) represent the peak current and scan rate, respectively. In Equation (2), \(b\) value is the slope of log \(i\) to log \(v\). Generally, when the \(b\) value is approximately 0.5, the RAC reaction is a diffusion-controlled process; however, if \(b\) is close to 1.0, it represents a surface-controlled process (Zhang et al., 2021). Fig. 2d shows that the \(b\) value was 0.846, demonstrating a surface control-dominant process of RAC oxidation on the surface of the CuNCs@CNFs, implying a strong adsorption of CNFs to RAC. To confirm RAC adsorption, CuNCs@CNFs/GCE, CNFs/GCE, and bare GCE were submerged into a 100 nM RAC solution, rinsed, transferred to PBS (0.01 M, pH 7.4) and finally the differential pulse voltammetry (DPV) was recorded. The high peak current signal of the
Based on the above results, the enhancement mechanism of CuNCs@CNFs toward RAC sensing was depicted in Scheme 1. RAC with phenolic hydroxyl groups could easily be adsorbed by the CNFs through π−π stacking, resulting in increased concentrations of RAC onto the surface of the electrode, simultaneously shorting the distance between RAC and Cu catalytic active sites on the surface of the CuNCs. Next, the captured RAC was oxidized via the catalytic properties of the CuNCs. The general oxidation process of RAC has been shown in previous reports (Rajkumar et al., 2013; Bai et al., 2014; Baytak et al., 2016). During the sensing process, porous CNFs provided numerous microfluidic channels needed to transport substances like electrolytes and reaction products while promote interfacial charge transfer. Based on the synergistic effects of the CuNCs and the CNFs, the CuNCs@CNFs expressed significantly enhanced RAC sensing performance.

3.3. RAC sensing-performance of the CuNCs@CNFs.

To evaluate the sensor’s sensitivity and potential quantitative applications, CuNCs@CNFs/GCE was used to detect RAC. DPV responses were recorded in 0.01 M PBS (pH 7.4) containing different RAC concentrations (Fig. 2e). The oxidation peak current increased with increasing RAC concentrations ranging from 0.1 to 800 nM. Using the linear equation: \( \Delta I (\mu A) = 0.048 \cdot C_{RAC} (nM) + 3.019 \) (\( R^2 = 0.998 \)) (Fig. 2f) the sensitivity was calculated at 679 \( \mu A \cdot M^{-1} \cdot cm^{-2} \). The amperometric technique (i-t) is considered a more sensitive detection method than DPV. Therefore, typical i-t curves were obtained by continuously adding specific concentrations of RAC at three different

Fig. 2. (a) CV curves towards RAC sensing of different modified electrodes, the electrolyte is 0.01 M PBS (pH 7.4) with 400 nM RAC. (b) Capacitive currents at 0.2 V as a function of scan rates for different electrodes. (c) CV curves of CuNCs@CNFs/GCE at different scan rates with 200 nM RAC in 0.01 M PBS (pH 7.4). (d) Functional relationship of log (peak current) vs log (scan rate). (e) DPV curves of CuNCs@CNFs/GCE at different concentrations RAC in 0.01 M PBS (pH 7.4). (f) Linear calibration curve of the peak currents versus RAC concentrations.
is the response time at 0.55 V. (b) Linear calibration curve of the response currents versus the low RAC concentrations corresponding to amperometric curve at 0.55 V. (c) Linear calibration curve of the response currents versus the high RAC concentrations corresponding to amperometric curve at 0.55 V. (d) and (e) Selectivity of CuNCs@CNFs/GCE sensor for RAC detection. Except for selectivity, the CuNCs@CNFs/GCE sensor was consistent with those determined by HPLC, indicating a high potential of this sensor in real sample RAC determination.

3.4. Real sample analysis

The practicality of the CuNCs@CNFs/GCE sensor was investigated by detecting RAC in pork, pork liver, chicken, duck, beef and mutton samples. Since RAC was not detectable in the samples (Table 2), different amounts of RAC were added to the sample solutions, and then were detected by both the CuNCs@CNFs/GCE sensor and HPLC to verify the accuracy of this method. The analysis results were summarized in Table 2, the average content of RAC at two spiked concentration levels was close to the content of RAC obtained from HPLC method. Meanwhile, the recoveries of CuNCs@CNFs/GCE sensor at two spiked concentration levels were in the range of 91.39 %–94.58 %, and the recoveries of HPLC ranged from 92.38 % to 95.62 %.

4. Conclusions

Herein, we proposed a novel electrochemical sensing platform based on CuNCs@CNFs for the ultrasensitive detection of RAC in food samples. CuNCs@CNFs were synthesized through the in situ carbothermic reduction of Cu2+ on porous carbon fibers. The distinct porous structure
the CNFs resulted in excellent electrocatalytic activity, and high sensing performance in RAC detection. In addition, the sensor was successfully used to determine RAC in different kinds of meat samples, and obtained satisfactory recoveries. This work provided a reliable and promising platform for the rapid ultrasensitive detection of RAC in foods.

CRediT authorship contribution statement

Yanan Lei: Data curation, Software, Writing – original draft. Yuhuan Zhang: Project administration, Formal analysis, Conceptualization, Writing – review & editing. Li Yuan: Data curation. Jianke Li: Project administration, Conceptualization, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1
Comparison of sensing performance presented in this work and previous works.

| Electrode | Detection methods | Linear range (µM) | LOD (nM) | Sensitivity (µA µM⁻¹ cm⁻²) | Ref. |
|-----------|-------------------|------------------|----------|--------------------------|------|
| G/GNRs /GCE | DPV 0.001–2.7 | 0.51 | 84 | (Bai et al., 2014) |
| AB/GCE | DPV 0.00296–5.92 | 1.6 | 101.351 | (Wang et al., 2015) |
| ATONP/GO/CNTs/GCE | DPV 0.01–0.24 | 3.3 | 0.558 | (Baytak et al. 2016) |
| BiTeO3@rGO/CNTs/SPCE | DPV 0.01–3.27 | 1.57 | – | (Muthumariyappan et al., 2019) |
| CDTS/ @Au/GCE | DPV 0.099–6.2 | 3.552 | – | (Song et al., 2020) |
| GO/GCE | DPV 0.074–2.96 | 56 | 5.888 | (Wu et al., 2012) |
| OMC/GCE | DPV 0.085–8.0 | 60 | – | (Yang et al., 2014) |
| NBC/GCE | DPV 0.1–1.75 | 41 | 0.416 | (Cao et al., 2021) |
| Poly taurine/ZrO2/GCE | DPV – 1.28 | 150 | 3.448 | (Rajkumar et al., 2013) |
| CNFs/GCE | i-t 1–100 | 340 | – | (Bai, 2022) |
| Au-Nps/AsME | DPV 0.1–380 | 50 | – | (Orooji et al., 2021) |
| TiO2-MWCNTs/3D | i-t 0.01–185 | 3.8 | – | (Keerthi et al., 2022) |
| CuNCs/CNTs/GCE | i-t 0.002–0.25 | 0.05 | 1641 | 806 | This work |

Table 2
Analysis results of RAC in samples obtained by i-t and HPLC method.

| Samples   | Added (nM) | i-t (nM) | Recovery | HPLC (nM) | Recovery |
|-----------|-----------|----------|----------|-----------|----------|
| Pork      | 0         | not detectable | not detectable | 46.10/92.20% | 46.78/93.57% |
| 50        | 92.73/92.73% | 94.12/94.12% | | | |
| Pork liver | 0         | not detectable | not detectable | 45.69/91.39% | 46.19/92.38% |
| 50        | 92.32/92.32% | 93.51/93.51% | | | |
| Chicken   | 0         | not detectable | not detectable | 46.19/92.38% | 47.00/94.00% |
| 100       | 93.12/93.12% | 94.63/94.63% | | | |
| Duck      | 0         | not detectable | not detectable | 47.29/94.58% | 47.81/95.62% |
| 100       | 94.41/94.41% | 95.13/95.13% | | | |
| Beef      | 0         | not detectable | not detectable | 46.78/93.57% | 47.09/94.18% |
| 100       | 93.22/93.22% | 95.91/93.91% | | | |
| Mutton    | 0         | not detectable | not detectable | 46.39/92.79% | 47.50/95.00% |
| 100       | 93.71/93.71% | 94.13/94.13% | | | |

1 Average value calculated from three determinations.

of the nanocomposite and the synergistic effects between the CuNCS and the CNFs resulted in excellent electrocatalytic activity, and high sensing performance in RAC detection. In addition, the sensor was successfully used to determine RAC in different kinds of meat samples, and obtained satisfactory recoveries. This work provided a reliable and promising platform for the rapid ultrasensitive detection of RAC in foods.

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