UNIVERSIDADE ESTADUAL DE MONTES CLAROS
Programa de Pós-Graduação em Botânica Aplicada
PPGBot

Sazonalidade, composição e armazenamento do pólen apícola de *Astronium urundeuva* (Anacardiaceae)

Érica Araújo Mendes Martins

Montes Claros - MG
Junho - 2024

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Dissertação apresentada à Universidade Estadual de Montes Claros como parte das exigências do Curso de Mestrado Acadêmico em Botânica Aplicada, área de concentração em Botânica Aplicada para a obtenção do título de Mestre.

Orientador(a): Prof. Dra. Hellen Cássia Mazzottini dos Santos

Coorientador(a): Prof. Dr. Leonardo Monteiro Ribeiro

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RESUMO

Sazonalidade, composição e armazenamento do pólen apícola de *Astronium urundeuva* (Anacardiaceae)

O pólen apícola é um produto rico em nutrientes e possui notável valor medicinal. Objetivou-se caracterizar a estrutura e composição química do pólen apícola de *Astronium urundeuva*, em função da sazonalidade e armazenamento. O pólen apícola foi coletado no norte de Minas Gerais, em quatro e três meses de dois anos subsequentes, no período de florescimento da espécie. Foram realizadas análises biométricas, de cor, de microscopia de luz e eletrônica de varredura, testes histoquímicos e análises bromatológicas, quantificação de fenólicos totais, flavonoides, antocianinas e carotenoides, avaliação da atividade antioxidante e caracterização do perfil de ácidos fenólicos. O percentual de grãos de pólen de aroeira nas amostras variou de 36,5 a 93,4%. As amostras de maio/23, junho/22 e 23 e julho/22 foram consideradas como monoflorais, pois apresentaram mais de 60% de grãos de pólen de aroeira e sem ocorrência de pólen acessório ou apresentaram mais de 80% de grãos de pólen de aroeira. Os lotes de pólen apícola com maior percentual de grãos de pólen de aroeira apresentaram predominância de pellets com coloração marron, com maior ocorrência em junho (83,9%). O pólen apícola de *A. urundeuva* mostrou-se uma boa fonte de proteínas e de compostos bioativos. A amostra com maior percentual de grãos de aroeira apresentou a maior concentração de fenólicos totais, flavonoides amarelos e carotenoides. O armazenamento diminui os teores dos compostos bioativos. Foram identificados os ácidos fenólicos: salicílico, vanílico, siríngico, gálico, ferúlico e cafeico nas amostras analisadas. Esses resultados confirmam que o pólen apícola de *A. urundeuva*, é um produto com alto valor nutricional, sendo considerado um superalimento e um alimento funcional.

Palavras-chave: aroeira; compostos bioativos; superalimento; alimento funcional.

ABSTRACT

Seasonality, composition and storage of bee pollen from *Astronium urundeuva* (Anacardiaceae)

Bee pollen is a product rich in nutrients and has notable medicinal value. The objective was to characterize the structure and chemical composition of *Astronium urundeuva* bee pollen, depending on seasonality and storage. Bee pollen was collected in the north of Minas Gerais, in four and three months of two subsequent years, during the species' flowering period. Biometric, color, light and scanning electron microscopy analyses, histochemical tests and bromatological analyses, quantification of total phenolics, flavonoids, anthocyanins and carotenoids, evaluation of antioxidant activity and characterization of the phenolic acid profile were carried out. The percentage of aroeira pollen grains in the samples ranged from 36.5 to 93.4%. The samples from May/23, June/22 and 23 and July/22 were considered as monofloral, as they presented more than 60% of aroeira pollen grains and no occurrence of accessory pollen or presented more than 80% of aroeira pollen grains. The bee pollen batches with the highest percentage of aroeira pollen grains showed a predominance of pellets with a brown color, with the highest occurrence in June (83.9%). *A. urundeuva* bee pollen proved to be a good source of proteins and bioactive compounds. The sample with the highest percentage of aroeira pollen grains had the highest concentration of total phenolics, yellow flavonoids and carotenoids. Storage reduced the levels of bioactive compounds. Phenolic acids were identified: salicylic, vanillic, syringic, gallic, ferulic and caffeic in the analyzed samples. These results confirm that bee pollen from *A. urundeuva* is a product with high nutritional value, being considered a superfood and a functional food.

Key words: aroeira; medicinal value; bioactive compounds; superfood; functional food.

SUMÁRIO

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Sazonalidade, composição e armazenamento do pólen apícola de *Astronium urundeuva* (Anacardiaceae)

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1. INTRODUÇÃO

O pólen apícola, resultado da aglutinação de grãos de pólen, efetuadas pelas abelhas, mediante adição de néctar e substâncias salivares, é considerado um superalimento, sendo fonte de proteínas, lipídios, minerais e compostos bioativos.[1] Esse produto também possui notável valor medicinal, por apresentar compostos com propriedades antioxidante, antimicrobiana, anti-inflamatório e anticarcinogênica.[2,3] Devido ao crescimento da demanda por produtos naturais e saudavéis, o número de pesquisas sobre as propriedades físico-químicas e funcionais do pólen apícola tem aumentado consideravelmente nos últimos anos.[4] No entanto, ainda são escassas as informações sobre o pólen apícola obtido a apartir de espécies arbóreas neotropicais, particularmento em ambientes semiáridos.

O pólen apícola possui variação estrutural, que está condicionada à sua composição botânica; podendo ser monofloral, com o predomínio de grãos de pólen de uma única espécie, ou multifloral, quando não há predominância de um tipo polínico.[5,6] A coloração pode ser a principal característica para a identificação do tipo de pólen apícola, o que é determinada pela diversidade de compostos químicos presentes nos grãos de pólen. As análises microscópicas são fundamentais para a identificação da origem botânica e quantificação dos grãos de pólen, os quais determinam as características morfológicas desse produto. [3,7,8].

A caracterização de aspectos físico-químicos do pólen apícola é essencial para o estabelecimento do padrão de qualidade para a sua aplicação no setor alimentício e medicinal, como observada nos estudos em que foram quantificados nutrientes, como proteínas, minerais, vitaminas e o potencial antioxidante de amostras de pólen apícola mono e multifloral.[9,10,11] Além da origem botânica, o perfil químico do pólen apícola pode ser influenciado por fatores ambientais, tais como clima, solo, temperatura, sazonalidade e radiação.[3,6,12] Adicionalmente,

o manejo pode comprometer a qualidade do pólen apícola nas fases de coleta, limpeza, desidratação, envase e armazenamento do produto fresco.[13,14,15,16]

Astronium urundeuva (M. Allemão) Engl., popularmente conhecido como aroeira, é uma árvore abundante nas florestas estacionais deciduais brasileiras, de grande importância econômica, pela utilização de sua madeira e por suas propriedades farmacológicas.[17] A utilização apícola de *A. urundeuva* tem se mostrado importante em regiões semiáridas do Brasil, uma vez que a espécie floresce na estação seca, época de escassez de recurso floral e o mel obtido é característico, com propriedades medicinais.[18,19] Os estudos apícolas com a aroeira estão concentrados na determinação da qualidade do mel, havendo grande lacuna de conhecimento acerca do pólen apícola dessa espécie.[18,20]

Informações detalhadas acerca da qualidade do pólen apícola são necessárias para incentivar o poder público e privado a subsidiar a coleta desse produto pelos apicultores, de modo a oferecer um alimento de alto valor nutricional.[4] Além disso, a produção do pólen apícola pode contribuir para a geração de trabalho e renda para o apicultor em vastas regiões semiáridas do Brasil e, de forma indireta, subsidiar a conservação de *A. urundeuva*, seja pelo favorecimento da polinização, ou pela preservação do ambiente, que é inerente à atividade.

Objetivou-se caracterizar a estrutura e a composição química do pólen apícola de *A. urundeuva*, a fim de responder às seguintes perguntas: i) Qual aspecto pode ser usado como marcador morfológico para a identificação do pólen apícola monofloral? ii) Qual é a composição química do pólen apícola? iii) Como a sazonalidade influencia a estrutura e composição do pólen apícola? iv) Quais alterações ocorrem na qualidade dos compostos químicos com o armazenamento?

2. MATERIAL E MÉTODOS

2.1. Local de estudo e coleta do material

O pólen apícola *in natura* de *A. urundeuva* foi coletado, em apiário localizado na zona rural do município de Januária, Minas Gerais, Brasil (15°29'52.5094"S; 44°30'13.4180"W) (Fig. 1A-D). As amostras foram coletadas, semanalmente, no período de maio a agosto de 2022 e março, maio e junho de 2023, e armazenadas em tubos Falcon, sob refrigeração, a -21 ºC, até o momento das análises.

A vegetação da região é caracterizada como transição entre Cerrado e Caatinga,[21] com floração abundante de aroeira durante a estação seca (Fig. 1B, D). A espécie é decídua, heliófita, seletiva xerófita e ocorre em agrupamentos densos.[19]

O clima é classificado como tropical úmido e seco do tipo AW, com estações chuvosa e seca bem definidas.[22] A temperatura máxima na região variou de 29,7 a 37,6 ºC, e a mínima variou de 12,5 a 23,1 ºC (Fig. 2). Não houve precipitação nos meses de maio a agosto de 2022 e junho e julho de 2023 (Fig. 2) (Instituto Nacional de Meteorologia – INMET, estação nº 83386).

2.2. Análise polínica e morfologia

As amostras de pólen apícola, obtidas semanalmente, foram agrupadas por mês. De cada lote formado foram pesados 4 g, em balança analítica (Shimadzu, ATX224), divididas em quatro subamostras, cujo pellets foram separados de acordo com a coloração em amarelo, branco e marrom, para obtenção do percentual de coloração do pólen apícola. De cada subamostra, foram mensurados comprimento e largura de 50 pellets, usando o Software ImageJ.

Para análise polínica, de cada lote formado foram pesados 2 g de pólen apícola, triturados com gral e pistilo e adicionado 13 mL de álcool 70% e deixado em repouso por 30

min. Uma gota da solução foi colocado sobre uma lâmina de microscopia e coberta com gelatina glicerinada e uma lamínula. A identificação e contagem foi realizada através da contagem de no mínimo 350 grãos de pólen, a documentação fotográfica foi feita usando câmera AxioCamMRC acoplado ao miscroscópio AxioVision LE (Zeiss, Oberkochen, Alemanha). As porcentagens de cada tipo polínico na amostra foram classificadas conforme a frequência em: pólen dominante (PD>45%), pólen acessório (PA de 15 a 45%), pólen isolado (PI<15%).[23]

A massa seca e o teor de água foram obtidos, a partir da secagem de quatro repetições de 2,0 g de pólen apícola, de cada lote, em estufa (Fanen, 315SE), a 105±5 ºC, até peso constante.[24]

2.3. Micromorfologia

Para a micromorfologia, pellets frescos, com diferentes colorações (branco, amarelo e marrom), foram pulverizados com 10 nm de ouro e analisado em microscópio eletrônico de varredura DSM 940A (Zeis, Cambridge, Reino Unido) a 15-20 kV.[25]

2.4. Anatomia e histoquímica

Para análise estrutural, pellets da amostra com mais 90% de grão de pólen de aroeira, foram fixados em solução de Karnovsky[26] por 12h, desidratados em série de etanol[27] e embebido em resina de hidroxietil-metacrilato (Leica Microsystems, Heidelberg, Alemanha).[28] Seções transversais, com 3 e 7 µm de espessura, foram obtidas usando micrótomo rotativo (Leica, Autocut, Alemanha), corados com azul de toluidina pH 4,7.[29]

Foram realizados testes histoquímicos, com reagente de Lugol[30] para detecção de amido; Xylidine Ponceau (XP)[31] para proteínas; Sudan Black[32] para lipídios totais; sulfato azul do Nilo[33] para lipídios neutros e ácidos; vermelho de rutênio[30] para pectinas; corifosfina,[34] sob luz UV, para pectinas; cloreto férrico[30] para compostos fenólicos; α-naftol e cloridrato de

dimetilparafenileno diamina (NADI)[35] para terpenos; vanilina clorídrica[36] para taninos; reagente de Dittmar[37] para alcaloides; p-dimetilaminocinamaldeído (DMACA)[38] para flavonoides. Cortes sem coloração foram usados como controle. A documentação fotográfica foi realizada usando câmera AxioCamMRc acoplada a microscópio AxioVision LE (Zeiss, Oberkochen, Alemanha).

2.5. Análises físicas e químicas

As análises físicas e químicas foram realizadas nos seguintes tratamentos: I) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 30 dias; II) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 240 dias; e III) pólen apícola desidratado e armazenado sob abrigo da luz, por 90 dias.

Foram pesados 30 g de pólen apícola em balança analítica (Shimadzu, ATX224), da amostra de junho de 2023, colocado em estufa, a 40 ºC até peso constante, para obtenção da amostra desidratada.[6]

A análise de cor foi determinada, em cada lote formado, a partir de dez pontos diferentes na amostra, com o auxílio do colorímetro Konica Minolta CR-400. Os parâmetros analisados foram L* (coeficiente de luz) que indicam valores que variam de 0 (totalmente preto) a 100 (totalmente branco); a* que varia de -80 (verde) a +100 (vermelho); b* que varia de -50 (azul) a +70 (amarelo). Os valores numéricos de a* e b* foram convertidos em C* (Chroma) e Ângulo Hue (ºH), de acordo com as seguintes equações: C* =$\sqrt{a2 + b2}$ e ºH= tg (a/b),[39] onde C* é o parâmetro quantitativo de intensidade de cor e ºH é o parâmetro qualitativo da cor,[40] de modo que o ângulo 0º indica a cor vermelha, 90º amarelo, 180º verde, 270º azul.[39]

Para determinar o teor de cinzas foi realizada a incineração em mufla (Analógica, AN1221), a 550 ºC,[41] em quatro repetições de 2,0 g de pólen apícola.

Para determinação de lipídios foram pesados 2,0 g de pólen apícola e transferidos para um cartucho de papel filtro previamente preparado. Os cartuchos foram transferidos para o aparelho extrator tipo Soxhlet (Marconi, MA044/8/50) e foram acoplados os reboliers devidamente pesados, a 105°C, contendo 70 mL de hexano (C_6H_{14}), ao extrator. Os cartuchos foram mergulhados no solvente, sob temperatura de 100 ºC, por 1 hora. O cartucho contendo a amostra foi suspendido para a região intermediária, por 1 hora, a 150°C. Após este período, a conexão intermediária foi trocada e iniciou-se a recuperação do solvente, durante 1 hora. Os reboliers foram desacoplados com os lipídios e o resíduo de hexano foi evaporado em estufa (Analógica, AN1221), a 105 ºC. Os reboliers foram resfriados em dessecador até temperatura ambiente e foram pesados.[41] A análise foi feita em três repetições.

O percentual de proteína foi analisado pelo método Micro Kjeldhal.[24] Foi pesado 0,3 g de pólen apícola e colocado em tubo de digestão, juntamente com 2,0 g de mistura digestora e 20 mL de ácido sulfúrico. A análise foi feita em quatro repetições. Foi realizada a digestão com temperatura inicial moderada a baixa até alcançar a temperatura de 350°C, continuou-se com o aquecimento até o clareamento da solução. Após resfriamento, foram adicionados 20 mL de água destilada na solução, sob agitação. Os tubos digestores com as amostras digeridas foram acoplados no conjunto de destilação e adicionou-se 20 mL de NaOH. Em um erlenmeyer foram adicionados 20 mL de ácido bórico e 2 gotas de solução de vermelho-de-metila. Adaptou-se o erlenmeyer ao conjunto de destilação para receber a amônia. Foi feita a destilação de arraste, mantendo o terminal do condensador mergulhado na solução receptora até a liberação total da amônia, o volume destilado foi de 100 mL. O erlenmeyer foi retirado e titulado com HCl 0,1N até a viragem do indicador (verde para rosa). Os volumes utilizados foram anotados.

Os carboidratos foram quantificados pela diferença das porcentagens médias de teor de água, proteínas, lipídeos e cinzas.[42]

Para determinar o teor de fenólicos totais, capacidade antioxidante pelo método de complexo de fosfomolibdênio e pelo método DPPH, foram feitos extratos conforme método descrito por Carpes.[43]

As amostras de pólen apícola (1 g cada) foram trituradas com o auxílio de gral e pistilo, extraídas com etanol 70% e homogeneizadas. Após 30 min o extrato foi filtrado e armazenado, sob refrigeração, até o momento das análises. As extrações e as análises foram feitas em triplicata.

O teor de compostos fenólicos totais foi determinado pelo método espectrofotométrico descrito por Whaterhouse[44]. Uma alíquota de 0,5 mL do extrato etanólico de pólen apícola foi adicionado aoa tubos de ensaio, em seguida foram adicionados 2,5 mL de solução de FolinCiocalteau 10% (v/v) e 2 mL de solução de carbonato de sódio 4% (p/v). Os tubos foram agitados em agitador vortex e mantidos em repouso, por 2 horas, no escuro. Após esse período, foram realizadas leituras em espectrofotômetro, a 720 nm (Shimadzu – UV 1280). A curva analítica foi preparada usando solução de ácido gálico (20-200 µg/mL) e os resultados expressos em g GAE/100g de amostra (GAE = equivalente em ácido gálico).

A atividade antioxidante, pelo método do complexo de fosfomolibdênio, foi descrito por Prieto et al..[45] Uma alíquota de 0,1 mL de extrato etanólico de pólen apícola foi colocada em tubos de ensaio e adicionados 3 mL de solução reagente (ácido sulfúrico 0,6 M, fosfato de sódio 28 mM e molibdato de amônio 4 mM). Os tubos foram fechados e incubados em banho-maria, a 95 ºC, por 90 minutos. Após o resfriamento, a leitura das absorbâncias foi mensurada em espectrofotômetro, no comprimento de onda de 695 nm (Shimadzu – UV 1280). A curva analítica foi preparada com solução de ácido ascórbico (1,95 a 500 µg), e os resultados foram expressos em g de AAE/100g de amostra (AAEs = equivalente de ácido ascórbico).

A atividade antioxidante realizada através do método de captura de radicais DPPH (2,2-difenil-1-picrilhidrazil) utilizou a metodologia desenvolvida por Brand-Willians et al..[46] Em tubos de ensaio, foi adicionado uma alíquota de 0,5 mL de extrato etanólico de pólen apícola e 0,3 mL de solução etanólica de DPPH 0,3 mM. O branco foi determinado a partir de 3,3 mL de etanol absoluto e 0,5 mL de extrato etanólico de pólen apícola e o controle negativo foi determinado a partir de 3,5 mL de etanol e 0,3 mL de solução etanólica de DPPH 0,3 mM. Os tubos de ensaio foram homogeneizados com agitador vortex e armazenados em temperatura ambiente e ausência de luz, por 30 minutos. Após esse período, foi feita a leitura da absorbância das amostras em espectrofotômetro, no comprimento de onda de 517 nm (Shimadzu – UV 1280).

Os valores de atividade antioxidante foram calculados via % média da atividade segundo a fórmula de Mensor et al.:[47]

$$\%AA = 100 - \frac{[(ABS\ amostra - ABS\ branco) * 100]}{\text{ABS controle}}$$

Onde: %AA=atividade antioxidante; ABS= leitura da absorbância.

A determinação de flavonoides amarelos e antocianinas foi realizada conforme a metodologia de Francis[48]. De cada amostra de pólen apícola, foi pesado 1 g e adicionado 20 mL de solução extratora (etanol 95%: HCl 1,5 N – 85:15 v/v). As amostras foram homogeneizadas e colocadas sob refrigeração (7 ºC) e abrigo da luz, por 16h. Após esse período, o extrato foi filtrado e as absorbâncias a 535 nm (antocianinas) e 374 nm (flavonoides amarelos) foram mensuradas. O conteúdo de flavonoides amarelos e antocianinas foi calculado de acordo com a equação abaixo.

$$\text{Flavonoides amarelos/antocianinas (g/100g)} = \frac{(\text{ABS x volume final}) * 10^4}{(peso\ amostra\ x\ \varepsilon^{1\%}_{1\ cm,535\ ou\ 374})}$$

Onde ABS= absorbância lida; $\varepsilon^{1\%}_{1cm,535}$=98,2 (mol/cm) (coeficiente de absortividade molar antocianinas); $\varepsilon^{1\%}_{1cm,374}$=76,6 (mol/cm) (coeficiente de absortividade molar flavonoides amarelos).

Para a determinação de carotenoides totais foi utilizada a metodologia de Carbonell-Capella et al..[49] De cada amostra de pólen apícola, foi pesado 1 g, triturada com auxílio de gral e pistilo, adicionado 5 mL de solvente extrator (hexano/acetona/etanol, 50:25:25, v/v/v) e centrifugada a 5,00 x g, por 10 min, a 4 ºC (Thermo Scientific – ST 16R). A camada sobrenadante do hexano contendo a coloração foi recuperada e transferida para um balão volumétrico de 25mL e o volume foi completado com hexano PA. Em seguida, foi realizada a leitura em espectrofotômetro no comprimento de onda 450 nm (Shimadzu – UV 1280). Os carotenoides totais foram determinados de acordo com a equação abaixo.

$$\text{Carotenoides totais (mg/100g)} = \frac{(\text{ABS x volume final}) * 1000}{(peso\ amostra\ x\ \varepsilon^{1\%}_{1\ cm,450})}$$

Onde: ABS= absorbância lida; $\varepsilon^{1\%}_{1cm,450}$ (coeficiente de absortividade molar) =2505.

Para determinação de compostos fenólicos por cromatografia gasosa acoplado a espectrometria de massa (CG-EM), foi pesado 0,15 g de pólen apícola de cada amostra e adicionado cerca de 1,5 mL de acetonitrila grau HPLC. As misturas foram homogeneizadas em vórtex, por um minuto, e mantidas em repouso, por 24 horas, a temperatura ambiente e no escuro. Os sobrenadantes foram recolhidos e 20 μL transferidos para *vials* internamente cônico. Em cada *vial* foram adicionados 100 μL BSTFA (com 1% de TMCS) e 60 μL de piridina. A mistura foi aquecida a 50 ºC, por 30 minutos, em banho de glicerina. Em seguida, o volume de reação foi transferido para um *vial* de injeção (2 mL) com *insert* para realização da análise cromatográfica.[50]

Todas as análises foram realizadas por CG-EM e os ácidos fenólicos (salicílico, vanílico, siríngico, gálico, ferúlico e cafeico) foram quantificados, por comparação de área com uma solução padrão na concentração de 1 mg L^{-1}, após derivatização.

As análises cromatográficas foram realizadas em cromatógrafo a gás da Agilent Technologies (GC 7890A) equipado com detector de massas (CG-EM) e coluna capilar SLB5-MS (Supelco, 30 m comprimento x 0,25 mm diâmetro interno x 0,25 μm espessura do filme). Hélio (99,99% de pureza) foi utilizado como gás de arraste a uma taxa de 1,0 mL min^{-1}. Utilizando um autoinjetor (CTC combiPaL), 1 μL da amostra foi injetada no cromatógrafo no modo *splitless*. O injetor *split/splitless* foi mantido a 290 °C.

A coluna cromatográfica inicialmente a 100 °C foi aquecida até 150 °C na taxa de 10 ºC min^{-1}. Em seguida, a temperatura foi elevada até 225 ºC, com taxa de 5 ºC min^{-1}, seguindo o aquecimento até 300 ºC com incremento de 20 ºC min^{-1}. A coluna foi mantida a 300 ºC, por 2 min. A temperatura da interface foi mantida a 280 ºC e a ionização realizada com impacto de 70 eV. A amplitude de varredura de *m/z* foi de 30 a 600 Da e no modo "sim" com monitoramento dos íons específicos para cada composto.[50] A identificação dos compostos presentes nos extratos foi realizada por comparação dos espectros de massas do banco de dados do aparelho (NIST 2.0).

2.6. Análise Estatística

Os dados quantitativos foram submetidos ao teste de normalidade de Shapiro-Wilk e ao teste de homogeneidade de variâncias de Levene. Análise de variância ou, alternativamente, o teste de Kruskal-Wallis foi utilizado e quando constatadas diferenças significativas entre os tratamentos, as médias foram comparadas pelo teste de Tukey ($P<0,05$) ou teste de Dunn ($P<0,05$), no caso de dados paramétricos ou não paramétricos, respectivamente.

3. RESULTADOS

3.1. Análise polínica e morfologia

As amostras de pólen apícola com maior percentual de grãos de pólen de aroeira apresentaram coloração escura (Fig. 3A), compostos por pellets com três cores: marrom (Fig. 3B), amarelo (Fig. 3C) e branco (Fig. 3C). O percentual de coloração dos pellets variou ao longo do tempo; lote de junho de 2022 apresentou a maior porcentagem de pellets marrons 83,9%, os meses de agosto de 2022 (1,4%) e março de 2023 (5,2%) apresentaram as menores porcentagens (Fig. 4).

A partir do espectro polínico foi possível determinar a origem botânica do pólen apícola (Tab. 1; Fig. 5). Grãos de pólen de aroeira apareceram em quase todas as amostras, variando de 36,5 a 93,4%, somente a amostra de março de 2023 não apresentou grãos de pólen de aroeira.

Os pellets apresentaram variação de 2,75 a 3,23 mm no comprimento (Fig. 6A) e a largura variou de 2,18 a 2,45 mm (Fig. 6B). A amostra com menor percentual de grãos de pólen de aroeira apresentarou pellets com maior dimensão (Fig. 6A-B). As médias de massa seca variaram de 78,52 a 86,0% (Fig. 6C), e teores de água variaram entre 14,00 a 21,48% (Fig. 6D).

3.2. Micromorfologia

Por meio da avaliação por miscroscopia de varredura foi possível constatar que os pellets são constituídos por numerosos grãos de pólen (Fig. 7). O pellet marrom (Fig. 7A-C) continha grãos de pólen de *A. urundeuva*, na forma desidratada com destaque para a ornamentação reticulada e para os colpos (Fig. 7C); houve a ocorrência de elemento figurado (Fig. 7B). No pellet amarelo (Fig. 7D-F), havia a presença de grãos de pólen de *A. urundeuva* e de Poaceae (Fig. 7 E), ambos na forma desidratada (Fig. 7F). No pellet branco (Fig. 7G-I), haviam grãos de pólen de *A. urundeuva* (Fig. 7H-I), *Richardia* (Fig. 7H-I), *Bauhinia* (Fig. 7H) e *Alternanthera* (Fig. 7I).

Grãos de pólen de *A. urundeuva* estavam aglutinados (Fig 8A), possuíam formato oblato esferoidal, com três colpos longos (Fig. 8B-C) e exina reticulada com lumens pequenos

(Fig. 8D). O pólen da *Alternanthera* (Fig. 9E) possuía 12 poros e exina com espículos diminutos (Fig. 9F). A *Bauhinia* (Fig. 9G) apresentou exina com presença de clavas (Fig. 9H). Em *Richardia* (Fig. 9I) a exina possuía espículos (Fig. 9J). O Tipo Poaceae apresentou um poro circular (Fig. 9K) e exina granulada (Fig. 9L).

3.3. Anatomia e histoquímica

O grão de pólen de aroeira apresentou uma variedade de compostos químicos, como evidenciado pela realização de testes histoquímicos. A exina é lignificada (Fig. 9A) e impregnada de compostos fenólicos (Fig. 9C, H), lipídios ácidos (Fig. 9D-E) e terpenos (Fig. 9F); a intina possui constituição péctica (Fig. 9A, I, J), associada a lipídios neutros (Fig. 9D-E), compostos fenólicos (Fig. 9C, H), flavonoides (Fig. 9G), e a terpenos (Fig. 9F). O protoplasto da célula vegetativa possui mucilagem (Fig. 9A), proteínas (Fig. 9B), amido (Fig. 9C), lipídios (Fig. 9D-E), terpenos (Fig. 9F), flavonoides (Fig. 9G), fenólicos (Fig. 9H) e pectina (Fig. 9I-J). O grão de pólen de aroeira não apresenta taninos e alcaloides.

3.4. Análises físicas e químicas

A coloração apresentou variação entre as amostras. O parâmetro L* (coeficiente de luz) foi maior nas amostras com 0% e 36,5% de grãos de pólen de aroeira e menor nas amostras com 73,3% e 93,4% de grãos de pólen de aroeira (Fig. 10A). O parâmetro a* (variando de verde a vermelho) foi menor nas amostras com73,3%; 79,8% e 93,4% de grãos de pólen de aroeira (Fig. 10B). Os parâmetros b* (variando de azul a amarelo), C* (Chroma, intensidade de cor) e hº (ângulo Hue) foram menores na amostra com 0% de grãos de pólen de aroeira e maiores nas amostras com 73,3 e 93,4% de grãos de pólen de aroeira (Fig. 10C-E).

O armazenamento alterou alguns parâmetros de coloração. O tratamento III apresentou aumento no parâmetro L* (Fig. 11A) e diminuição no parâmetro a* (Fig. 11B), o tratamento II

teve diminuição no parâmetro a* (Fig. 11B). O parâmetro hº apresentou acréscimo nos tratamentos II e III. Os parâmetros b* e C* não apresentaram diferenças significativas entre amostras, pelo teste de Tukey, apresentando médias de 30,01 (P=0,538) e 30,30 (P=0,677), respectivamente.

As amostras apresentaram teores de cinzas na faixa de 2,93 a 4,65% (Fig. 12A), lipídios entre 2,82 a 5,03% (Fig. 12B) e as proteínas variaram de 17,90 a 25,54% (Fig. 12C), com diferenças significativas entre eles. Os carboidratos não tiveram diferença significativa, apresentando média de 52,66% (P=0,121).

A análise química do pólen apícola, em função da sazonalidade mostrou variação na composição de fenólicos totais (Fig. 13A), flavonoides amarelos (Fig. 13B), antocianinas (Fig. 13C), carotenoides (Fig. 13D), e atividade antioxidante pelo método do complexo fosfomolibdênio (Fig. 13E). A amostra sem grãos de pólen de aroeira apresentou a menor quantidade de fenólicos totais, antocianinas e carotenoides (Fig. 13A, C e D) e a maior atividade antioxidante pelo método do complexo fosfomolibdênio (Fig. 13E). A amostra com 84,2% de grãos de aroeira apresentou a maior concentração de fenólicos totais (Fig. 13A), flavonoides amarelos (Fig. 13B) e carotenoides (Fig. 13D). A atividade antioxidante, pelo método de DPPH, não teve diferença significativa, apresentando média de 91,2% (P=0,132).

A amostra com 84,2% de grãos de pólen de aroeira foi testada em relação ao armazenamento. A amostra conservada, sob refrigeração, por 240 dias, e a desidratada, conservada, por 90 dias, apresentaram uma diminuição nos valores de fenólicos totais (Fig. 14A), flavonoides amarelos (Fig. 14B), antocianinas (Fig. 14C) e carotenoides (Fig. 14D), em comparação a amostra conservada sob refrigeração, por 30 dias. A atividade antioxidante pelo método do complexo de fosfomolibdênio e pelo método DPPH não tiveram diferenças

significativas entre as amostras, apresentando médias de 29,54 g/100g AAEs (P=0,05) e 92,80% (P=0,44), respectivamente.

Os cromatogramas referentes às análises de ácidos fenólicos (salicílico, vanílico, siríngico, gálico, ferúlico e cafeico) das amostras estão apresentadas na Figura. 15, e os valores encontrados estão apresentados na Tabela 2.

A amostra sem grãos de pólen de aroeira apresentou as maiores quantidades de ácidos vanílico, siríngico, gálico, férulico e cafeico, e a amostra com 79,8% apresentou a maior concentração de ácido salicílico. A desidratação da amostra de 84,2% elevou a concentração dos ácidos salicílico, vanílico, siríngico, gálico e ferúlico, em relação a amostra congelada.

4. DISCUSSÃO

Nossos dados indicaram que o pólen apícola monofloral de *A. urundeuva* foi constituído por mais de 70% de grãos de pólen de aroeira. O produto apresenta características morfológicas que podem ser associadas com a fenologia e sazonalidade da espécie e, pode ser considerado um superalimento, pois possui composição química rica em nutrientes essencias e atividade antioxidante, o método e período de armazenamento são fatores que comprometem a de qualidade do pólen apícola.

Marcador morfológico e identificação do pólen apícola monofloral

A coloração e quantidade de pellets podem ser um marcador morfológico para a identificação do pólen apícola monofloral de *A. urundeuva*. O pólen apícola é constituído por pellets de diferentes colorações, o que demonstra a diversidade botânica do produto. O maior percentual de pellets com coloração marrom foi observado na amostra com maior percentual de grãos de pólen de *A. urundeuva*. A amostra com o menor percentual de grãos de pólen de aroeira e a amostra que não continha grãos de pólen de aroeira apresentaram, também, pellets

de coloração marrom, mas em quantidades diminutas. Pólen apícola de uma mesma espécie botânica pode apresentar cores diferentes, e pólen apícola de espécies botânicas diferentes podem apresentar a mesma cor.[4] Na área de estudo, a presença de pellets de coloração marrom indicou a presença de grãos de pólen de aroeira, e quanto mais pellets marrons, mais grãos de pólen de aroeira estavam presente no pólen apícola.

As medidas de comprimento e largura dos pellets de pólen apícola podem ser influenciadas pela coleta realizada pelas abelhas e pela grade de retenção, presente nos coletores, que possuem orifícios de 4,3 a 5 mm, que permite a passagem das abelhas, mas impõe a separação dos pellets presos em suas pernas.[51] Estudo realizado em amostra de pólen apícola da República Tcheca encontrou valores médios para comprimento de 2,7-3,5 mm e para largura 3,3-3,8 mm,[3] resultado semelhante ao encontrado no presente trabalho.

A amostra que não apresenta grãos de pólen de aroeira apresentou um maior teor de água, o que pode ser explicado pela diversidade de tipos polínicos, que podem possuir uma maior a capacidade de retenção de água.[52] A amostra, em questão, foi coletada no mês em que houve precipitação, o que pode indicar uma possível correlação.

Composição química do pólen apícola em função da sazonalidade

As amostras analisadas apresentaram variações na composição química, em função a sazonalidade. A origem botânica é um fator interferente nas concentrações dos compostos químicos, pois as amostras estudadas tiveram diferenças no espectro polínico. A cor das amostras de pólen apícola é afetada pela composição química dos grãos de pólen.[4] A amostra com maior percentual de grãos de pólen de aroeira apresentou-se mais escura, com tendência ao vermelho e ao amarelo e um dos maiores valores de intensidade de cor. A concentração de compostos químicos foi correlacionada com a cor do pólen apícola no trabalho realizado por De Melo et al..[53]

As amostras de maio/23, junho/22 e 23 e julho/22 foram consideradas como monoflorais, pois apresentaram mais de 70% de grãos de pólen de aroeira e sem ocorrência de pólen acessório ou apresentaram mais de 80% de grãos de pólen de aroeira. Esse critério também foi utilizado por outros pesquisadores para classificação de pólen monofloral.[5,23,54] As amostras de maio/22, agosto/2022 e março/2023 foram consideradas multiflorais.

As amostras que continham grãos de pólen de aroeira exibiram valores entre 22-26% de proteína. Resultado semelhante foi encontrado no estudo realizado com amostra de pólen apícola com frequência de 84% de grãos de pólen de *Eucalyptus*, do Sul do Brasil, que apresentou teor de proteína de 25%.[11] Em amostras de pólen apícola monofloral de *Cocos nucifera*, do Sul da Bahia, foram encontrados valores de proteínas entre 13-15%.[55] O teor de proteína encontrado no pólen apícola é maior que o teor encontrado em outros alimentos como ovos (12,8%) ou carne de porco (14,2%).[10]

A legislação brasileira determina que o pólen apícola *in natura* tenha no máximo 30% de umidade e 4% de cinzas e no mínimo 1,8% de lipídios e 8% de proteínas.[1] Os resultados encontrados no presente estão de acordo com os valores preconizados pela legislação, exceto nas amostras de 36,5 e 66,2% de grãos de pólen de aroeira que apresentaram teor de cinzas acima do limite permitido.

Os compostos bioativos analisados, neste trabalho, exibiram diferenças significativas em relação ao percentual de grãos de pólen de aroeira, a amostra com maior percentual apresentou também a maior concentração de fenólicos totais, flavonoides amarelos, e carotenoides, indicando o grande potencial nutricional do pólen apícola monofloral de *A. urundeuva*. Os compostos bioativos encontrados nos alimentos são resultado do metabolismo secundário das plantas e desempenham papel importante na resposta da defesa contra patógenos e na atração de polinizadores. Esses compostos apresentam uma variedade de efeitos benéficos

para os humanos e despertam interesse da indústria de alimentos, farmacêutica e cosmética, e são objetos de estudos de diversos trabalhos.[53,56,57,5,59]

A atividade antioxidante foi avaliada por dois métodos distinto, ambos apresentaram resultados satisfatórios, a amostra que não apresenta grãos de pólen de aroeira exibiu maior atividade antioxidante pelo método do complexo de fosfomolibdênio, situação que pode estar relacionada a uma concentração maior de ácido cafeico em relação as outras amostras; pelo método de DPPH as amostras não apresentaram diferenças significativas. Os resultados encontrados nesse trabalho estão de acordo com os estudos realizados em amostras multiflorais, do Sul do Brasil, e em amostras de pólen apícola monofloral da Republica Tcheca, onde foram encontrados valores de 94% e 25 a 93%, respectivamente. [5,3] Amostras de pólen apícola mono e multiflorais da Turquia, apresenta apresentaram dados contrastantes, com valores variando entre 4 e 23%.[54]

Na área estudada, o pólen apícola monofloral de aroeira foi encontrado no período de seca, nos meses de maio, junho e julho, época de floração da espécie, o pico da floração ocorreu no mês de junho, tendo as amostras deste mês o maior percentual de grãos de pólen da espécie. Mais estudos são necessários para caracterização do pólen apícola de *A. urundeuva* de outras regiões, para avaliação da sua estrutura e composição sobre influência de condições abióticas e adversas.

Qualidade dos compostos químicos e armazenamento

Os métodos de armazenamento, comumente implementados pelo apicultor, alteram a qualidade dos compostos químicos do pólen apícola. A desidratação fez com que a amostra ficasse mais escura e os métodos de armazenamento, avaliados no presente trabalho, provocaram uma redução significativa dos teores de fenólicos totais, flavonoides amarelos, antocianinas e carotenoides. Essas alterações podem ser devidas as reações de oxidação dos

compostos químicos, principalmente os compostos fenólicos.[4] Entretanto, a atividade antioxidante, pelos dois métodos analisados, não apresentou diferenças entre os valores. Resultados semelhante foi encontrado por Anjos et al.,[16] que quantificou fenólicos totais e flavonoides totais em amostras de pólen apícola, mono e multiflorais, congelados e desidratados. Melo e Almeida-Muradian[9] avaliaram os teores de carotenoides totais e outros compostos bioativos, em relação ao pólen fresco e desidratado, e todas as amostras avaliadas tiveram redução nos teores dos compostos. Arruda et al.[60] quantificaram vitaminas do complexo B, em relação ao processamento e tempo de armazenamento, e encontrou valores variados, alguns teores tiveram aumento e outros apresentaram uma diminuição, com o decorrer dos dias.

O pólen apícola é rico em polifenóis, como flavonoides e ácidos fenólicos, e como mencionado anteriormente, a concentração de tais substâncias depende da origem botânica. [61] Os cromatogramas exibiram a detecção de vários compostos nas amostras de pólen apícola e a variação destes ao longo do tempo de coleta e também do processo de desidratação. Além dos compostos identificados, o cromatograma indica uma diversidade de compostos que necessitam de maiores estudos.

O presente trabalho demonstrou que o pólen apícola monofloral de aroeira apresentou maior número de pellets de coloração marrom e a quantidade destes representou um maior percentual de grãos de pólen de aroeira, na área de estudo e no período de floração da espécie. As amostras apresentaram diversificada composição química em relação a sazonalidade, com as amostras monoflorais de *A. urundeuva* apresentando as maiores frações de proteínas e compostos bioativos, como fenólicos totais, flavonoides amarelos, antocianinas e carotenoides, além de exibirem atividade anitoxidante. Os métodos de armazenamento (congelamento e desidratação) resultaram na diminuição dos teores de fenólicos totais, flavonoides amarelos, antocianinas e carotenoides, entretanto a atividade antioxidante não teve alteração. Diante de

todos os resultados encontrados o pólen apícola, especialmente o monofloral de *Astronium urundeuva*, pode ser considerado um superalimento ou alimento funcional.

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6. TABELAS

Tabela 1 - Espectro polínico e percentual de grãos de pólen das amostras de pólen apícola. Pólen dominante (PD>45%); pólen acessório (PA de 15 a 45%), pólen isolado (PI<15%).

Imagem (Fig. 5)	Tipo polínico	Família	Período de coleta						
			2022				2023		
			mai	jun	jul	ago	mar	mai	jun
D	*Alternanthera*	Amaranthaceae	17,5	0,1	3,2	0,3	0,5	3,3	4,5
A-C	*Astronium urundeuva*	Anacardiaceae	66,2	93,4	73,3	36,5		79,8	84,2
-	*Schinopsis brasiliensis*	Anacardiaceae		0,7	1,1				
-	*Bidens*	Asteraceae						0,2	0,1
S	*Vernonia*	Asteraceae				2,2	5,0		
O	*Ipomea*	Convolvulaceae					3,0		
-	*Convulvulaceae*	Convolvulaceae	0,2				0,2		
-	*Merremia*	Convolvulaceae					14,2		
-	*Croton*	Euphorbiaceae	0,8						
-	*Ricinus*	Euphorbiaceae				0,6			
-	*Euphorbiaceae sp.*	Euphorbiaceae		1,1	6,3	44,4		1,3	
H-I	*Bauhinia*	Fabaceae	1,1	2,0	5,0	7,5			0,5
-	*Bauhinia forficata*	Fabaceae					35,8		
K-L	*Ceiba*	Fabaceae		0,2	6,7	3,0		1,9	8,8
J	Tipo *Mimosa*	Fabaceae					7,5		
-	*Senegalia*	Fabaceae		0,1		0,7		1,0	
-	Fabaceae	Fabaceae						0,8	
P	*Hyptis*	Lamiaceae					0,7		
M	*Malvastrum*	Malvaceae		0,1			0,5		
-	*Melochia*	Malvaceae							
-	*Sida*	Malvaceae					0,5		
Q-R	*Pseudobombax*	Malvaceae	0,9			2,2			
-	Melastomataceae	Melastomataceae				2,0			
N	*Eucalyptus*	Myrtaceae				0,3			
-	*Passiflora*	Passifloraceae					0,5		
G	*Poaceae*	Poaceae	6,8				15,4	6,0	0,9
-	*Zea mays*	Poaceae					3,7		
E-F	*Richardia*	Rubiaceae	6,2	1,0			12,4	5,8	0,6
T	*Serjania*	Sapindaceae				0,3			
-	*Dilodendron bipinnatum*	Sapindaceae	0,3	1,3	1,3				0,5

Tabela 2. Concentração de ácidos fenólicos nas amostras de pólen apícola, em µg/Kg.

% de grãos de pólen de aroeira	Ácidos fenólicos					
	Salicílico	Vanílico	Siríngico	Gálico	Ferúlico	Cafeico
0 (II)	83,21	161,70	19,64	340,20	361,10	1133,00
79,8 (II)	273,2	91,29	8,76	68,40	32,26	63,55
84,2 (II)	78,98	22,24	3,57	12,83	11,87	29,45
84,2(III)	126,4	23,09	6,99	19,82	13,73	13,62

Tratamento das amostras: II) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 240 dias; e III) pólen apícola desidratado e armazenado sob abrigo da luz, por 90 dias.

7. FIGURAS

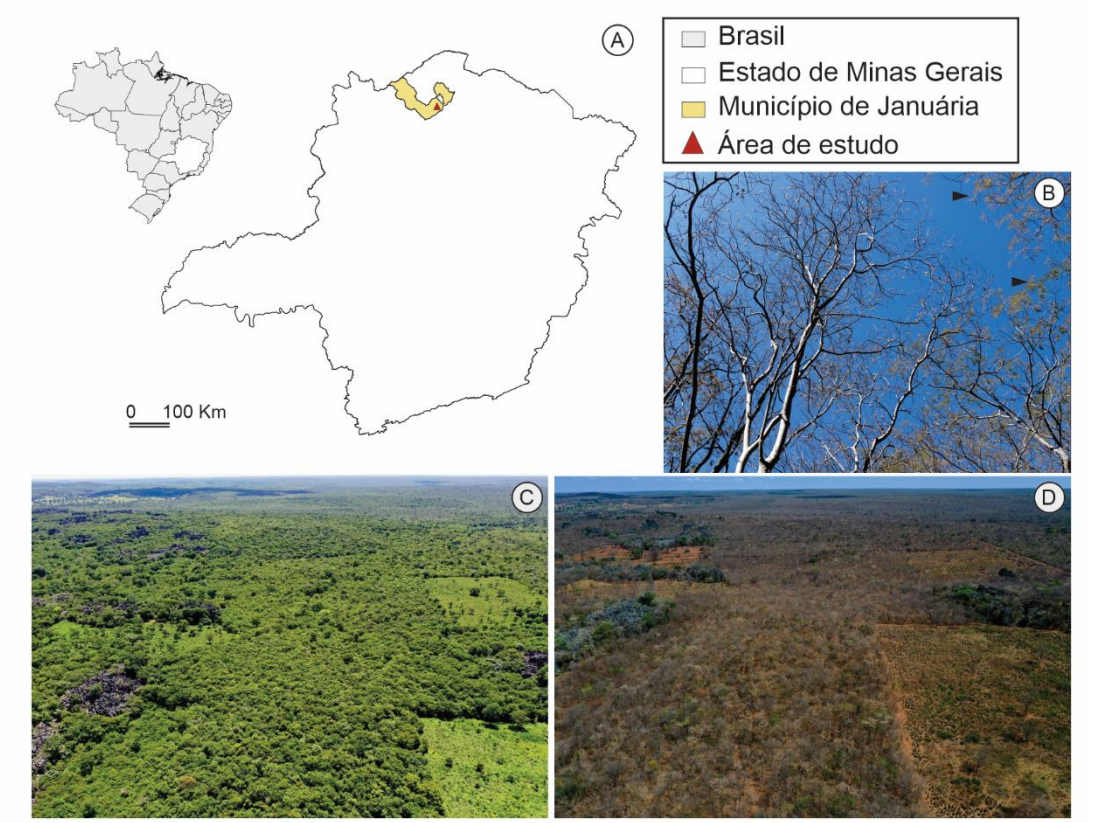


Fig. 1. Área de coleta do pólen apícola, em Minas Gerais, Brasil (A). Árvores de *Astronium urundeuva* no período de floração (ponta de seta) (B). Vegetação da área de estudo na estação chuvosa (C). Vegetação no período de floração, estação seca (D).

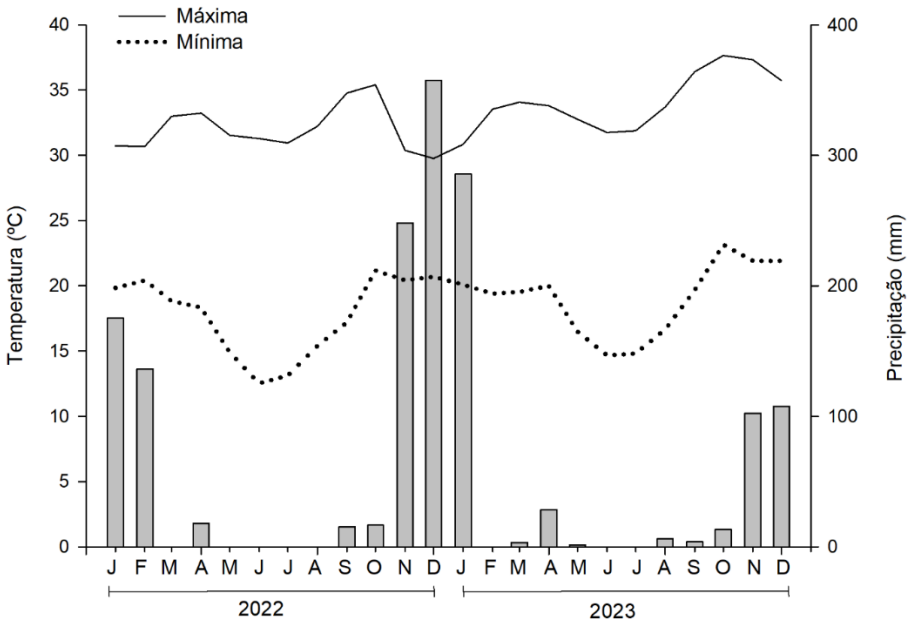


Fig. 2. Temperatura e precipitação obtidas da Estação Climática de Januária, no período de janeiro de 2022 a dezembro de 2023.

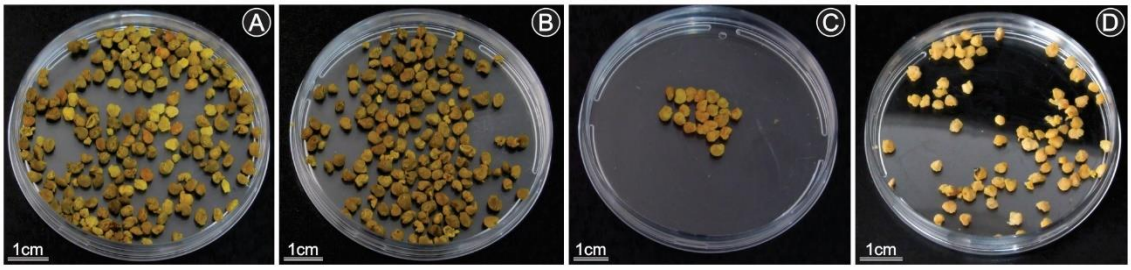


Fig. 3. Variação de cor dos pellets na amostra de pólen apícola. (A) Pellets com várias cores. (B) Pellets com coloração marrom. (C) Pellets com coloração amarelada. (D) Pellets como coloração branca.

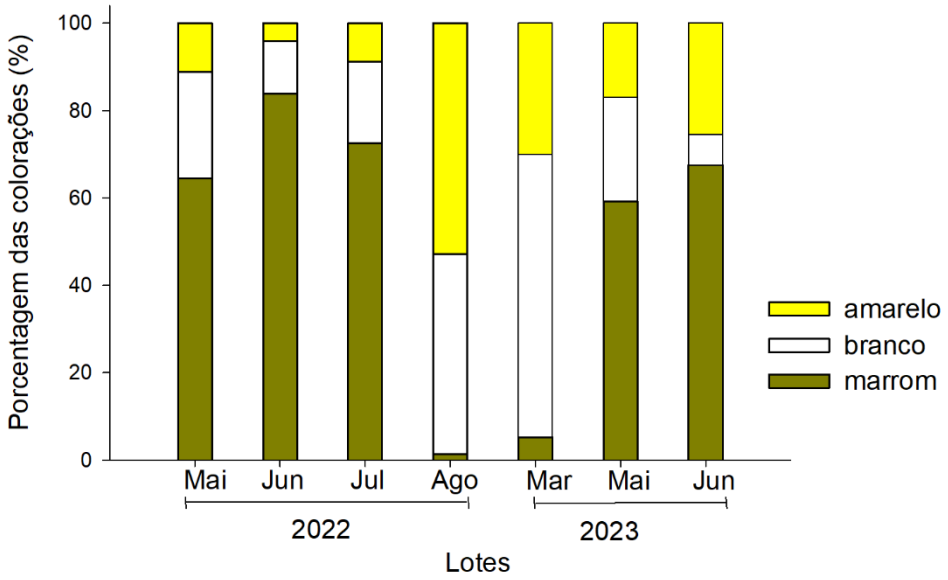


Fig. 4. Percentual de coloração dos pellets nas amostras de pólen apícola.

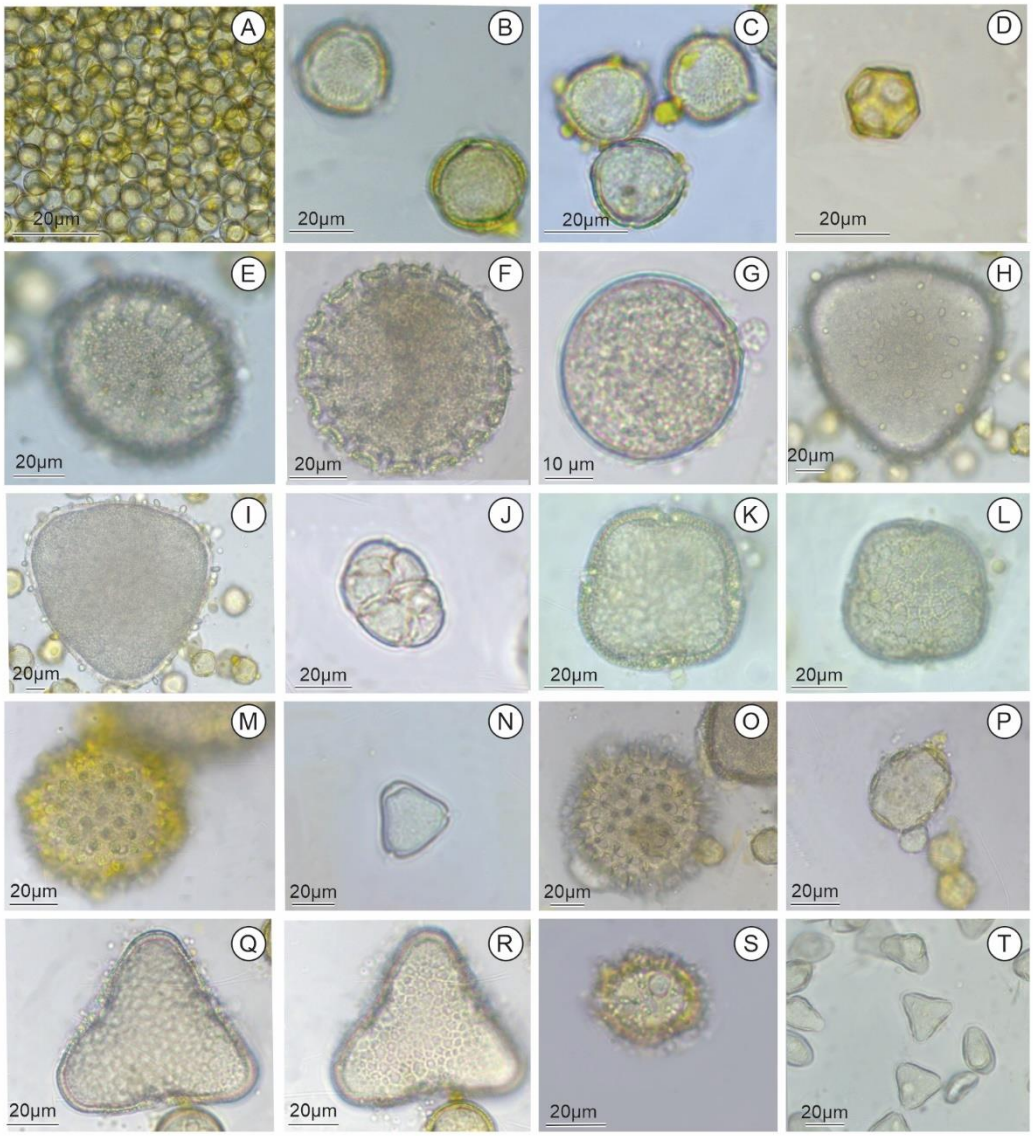


Fig. 5. Tipos polínicos presentes nas amostras de pólen apícola. (A-C) *Astroniun urundeuva* (aroeira). (D) *Alternanthera*. (E-F) *Richardia*. (G) Poacaeae. (H-I) *Bauhnia*. (J) Tipo *Mimosa*. (K-L) *Ceiba*. (M) *Malvastrum*. (N) *Eucalyptus*. (O) *Ipomea*. (P) *Hyptis*. (Q-R) *Pseudobombax*. (S) Vernonia. (T) *Serjania*.

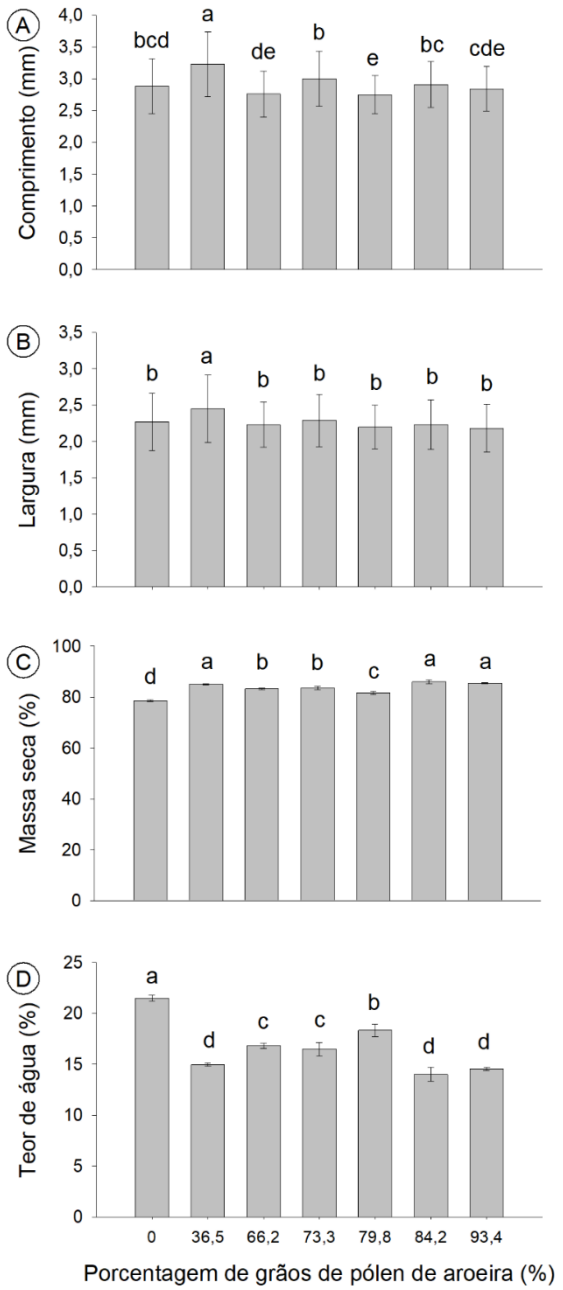


Fig. 6. Valores médios de comprimento (A), largura (B), massa seca (C) e teor de água (D) nas amostras de pólen apícola. Barras verticais representam o desvio padrão das médias; letras diferentes indicam diferença significativa pelo teste de Dunn (A-B) e teste de Tukey (C-D) ($P<0,05$).

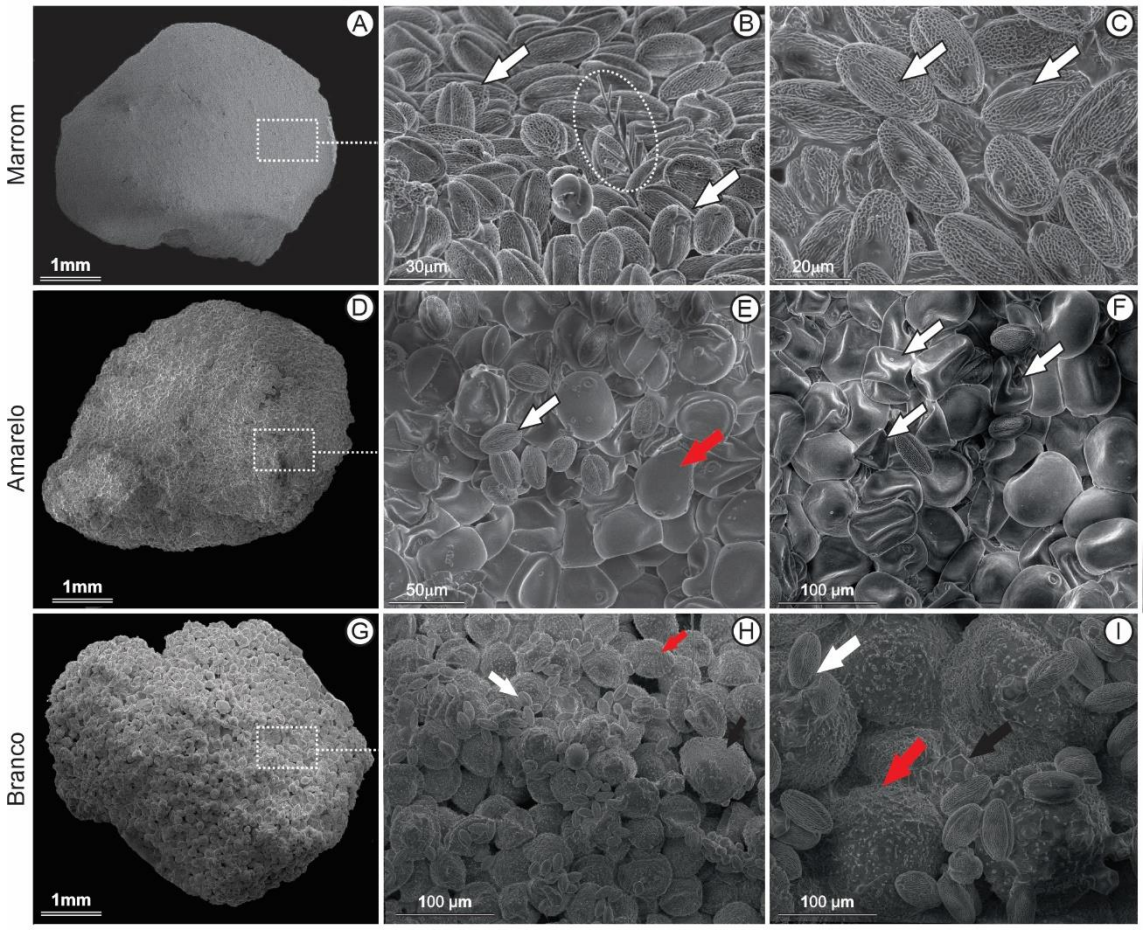


Fig. 7. Micrografias eletrônica de varredura dos pellets marrom, amarelo e branco da amostra de pólen apícola. (A) Vista geral do pellet marrom com superfície ligeiramente lisa. (B) Grãos de pólen *A. urundeuva* (setas), com destaque para elemento figurado (pontilhado). (C) Vista da ornamentação reticulada do pólen de *A. urundeuva* com colpos alongados (setas) na forma desidratada. (D) Vista geral do pellet amarelo com superfície irregular. (E) Presença de vários tipos polínicos, *A. urundeuva* (seta branca) e Poaceae (seta vermelha). (F) Grão de pólen do Tipo Poaceae (setas) desidratado. (G) Vista geral do pellet branco com superfície irregular. (H) Presença de vários tipos polínicos, *A. urundeuva* (seta branca) *Richardia* (seta vermelha) e *Bauhinia* (seta preta). (I) Grão de pólen de *A. urundeuva* (seta branca), *Richardia* (seta vermelha) e *Alternanthera* (seta preta).

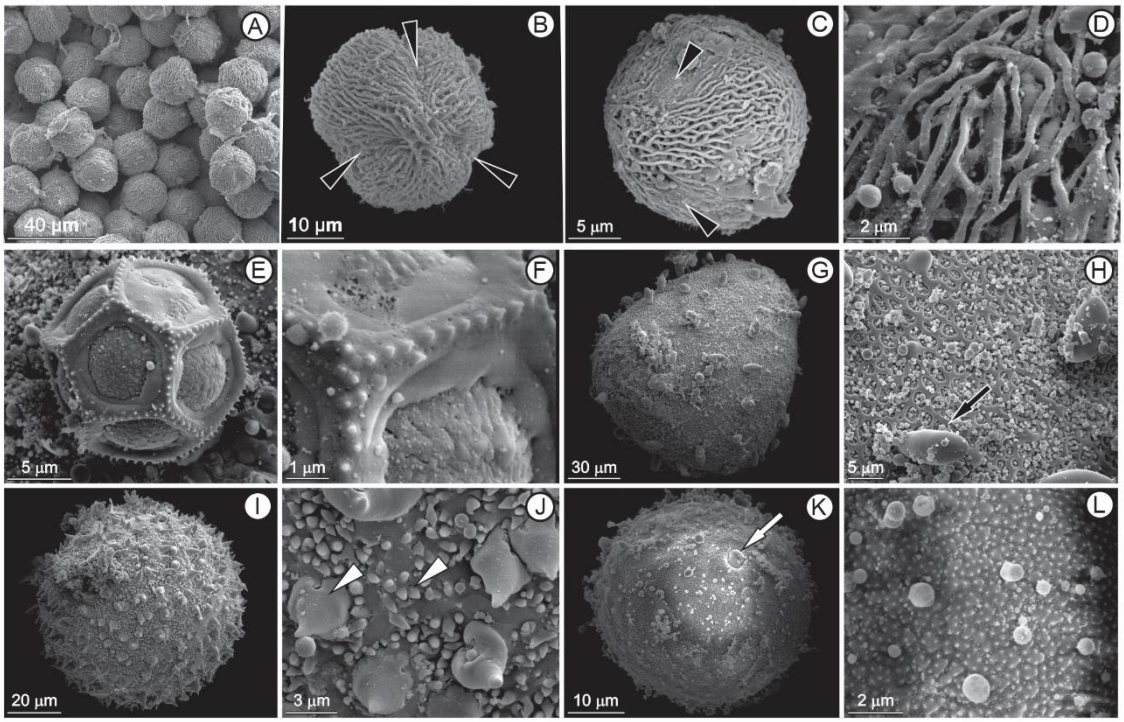


Fig. 8. Micrografias eletrônica de varredura dos grãos de pólen presentes na amostra de pólen apícola. (A-D) Grão de pólen de *A. urundeuva*, (A) Grãos de pólen de *A. urundeuva* aglutinados. (B - C) Grãos de pólen com diferentes níveis de hidratação. As pontas de seta mostram os colpos alongados do grão de pólen de *A. urundeuva*. (D) Detalhe da ornamentação reticulada da exina com lumens pequenos e profundos. (E-F) Tipo *Alternanthera*, apresenta 12 poros. (F) Detalhe dos pequenos espículos presente na exina. (G-H) Tipo *Bauhinia*. (H) Detalhe para detalhe a presença de clavas na superfície (seta). (I-J) Tipo *Richardia*. (J) Detalhe da ornamentação na exina, com presença de espículos (pontas de seta). (K-L) Tipo Poaceae, com poro circular (seta). (L) Detalhe da exina granulada.

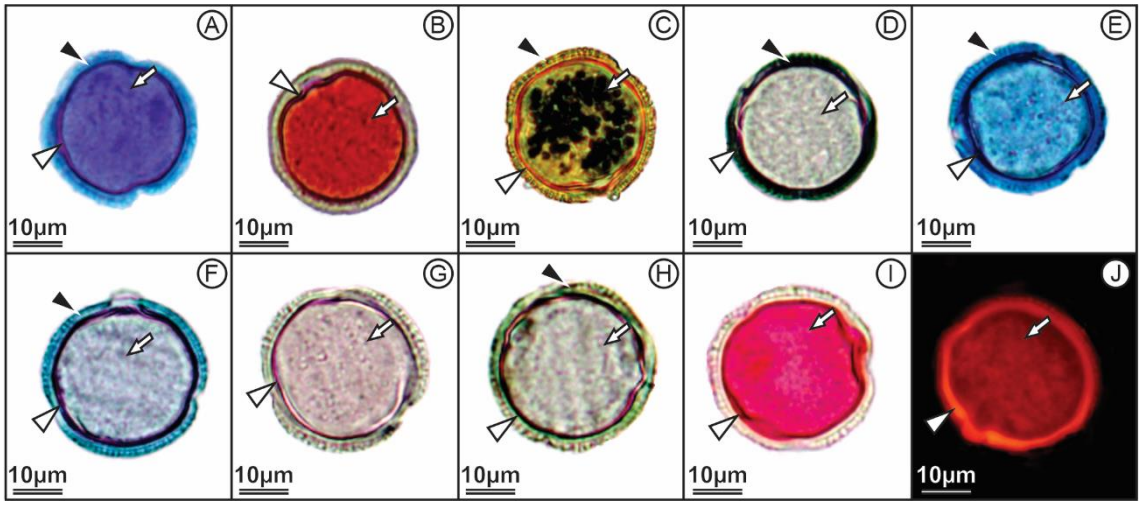
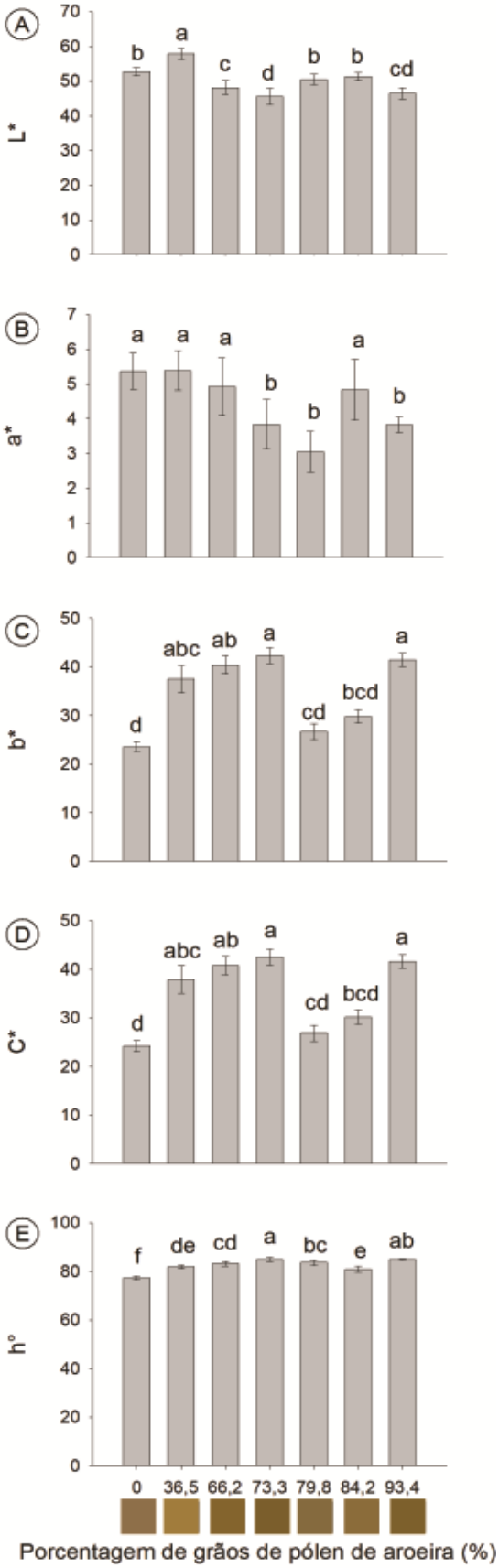


Fig. 9. Grãos de pólen de *Astronium urundeuva* (A-J). (A) Grão de pólen apresentando exina lignificada, intina péctica e protoplasto rico em mucilagem e pectina. (B) Presença de proteínas na intina e no protoplasto. (C) Grãos de amido no protoplasto e compostos fenólicos na exina e intina. (D) Presença de lipídios na exina, intina e protoplasto. (E) Lipídios neutros na intina e ácidos na exina e no protoplasto. (F) Presença de terpenos na exina, intina e protoplasto. (G) Presença de flavonoides na intina e no protoplasto. (H) Presença de compostos fenólicos na exina, intina e protoplasto. (I-J) Presença de pectina na intina e no protoplasto. Ponta de seta preta: exina; ponta de seta branca: intina; seta: protoplasto.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Porcentagem de grãos de pólen de aroeira (%)

Fig. 10. Valores dos parâmetros de cor: L*, a*, b*, C* e hº das amostras de pólen apícola com diferentes porcentagens de grãos de pólen de *Astronium urundeuva* em relação a sazonalidade. (A) L* (coeficiente de luz, variando de 0 [totalmente preto]) a 100 [totalmente branco]). (B) a* (variando de -80 [verde] a +100 [vermelho]); b* (variando de -50 [azul] a +70 [amarelo]). (C) b* (variando de -50 [azul] a +70 [amarelo]). (D) C* (Chroma, parâmetro de intensidade de cor). (E) hº (ângulo Hue, parâmetro qualitativo de cor, variando de 0º [cor vermelha], 90º [cor amarela], 180º [cor verde] e 270º [cor azul]). Barras verticais representam o desvio padrão das médias, letras diferentes indicam diferença significativa pelo teste de Dunn (C – D) ($P<0,05$) e pelo teste de Tukey ($P<0,05$) (A, B e E). Parâmetros de cor com visual RGB obtido do site: <https://colordesigner.io/convert/labtorgb>.

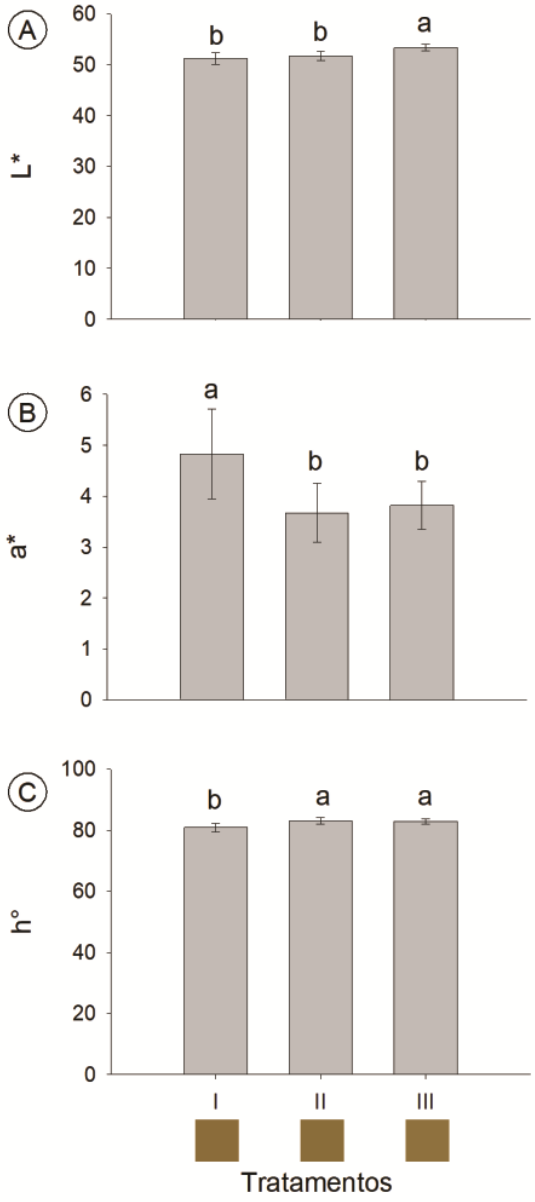

Fig. 11. Valores dos parâmetros de cor: L*, a* e hº da amostra com 84,2% de grãos de pólen de *Astronium urundeuva* em relação ao armazenamento. (A) L* (coeficiente de luz, variando de 0 [totalmente preto]) a 100 [totalmente branco]). (B) a* (variando de -80 [verde] a +100 [vermelho]); b* (variando de -50 [azul] a +70 [amarelo]). (C) hº (ângulo Hue, parâmetro qualitativo de cor, variando de 0º [cor vermelha], 90º [cor amarela], 180º [cor verde] e 270º [cor azul]). Barras verticais representam o desvio padrão das médias, letras diferentes indicam diferença significativa pelo teste de Tukey (P<0,05). Tratamentos: I) pólen apícola *in natura*

mantido sob refrigeração, a -21 ºC, por 30 dias; II) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 240 dias; e III) pólen apícola desidratado e armazenado sob abrigo da luz, por 90 dias. Parâmetros de cor com visual RGB obtido do site: <https://colordesigner.io/convert/labtorgb>.

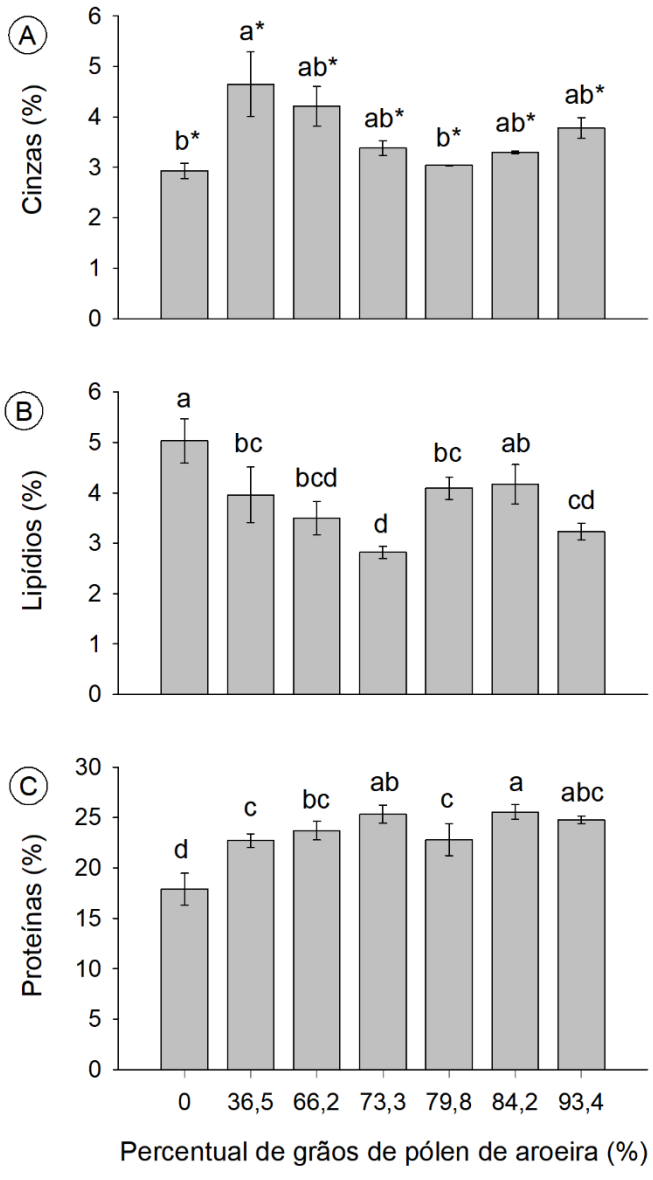

Fig. 12. Composição química das amostras de pólen apícola com diferentes porcentagens de grãos de pólen de *Astronium urundeuva* (A-C). Barras verticais representam o desvio padrão

das médias, letras diferentes indicam diferença significativa pelo teste de Dunn (P<0,05) (A) e pelo teste de Tukey (P<0,05) (B-C).

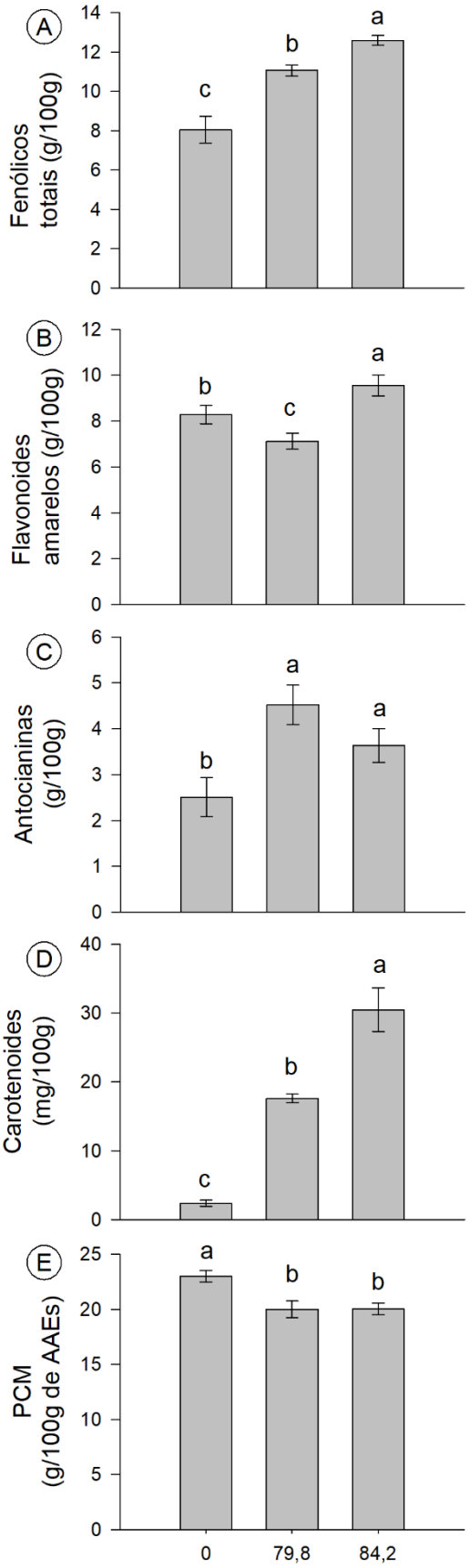
A
Fenólicos totais (g/100g)
B
Flavonoides amarelos (g/100g)
C
Antocianinas (g/100g)
D
Carotenoides (mg/100g)
E
PCM (g/100g de AAEs)
0
79,8
84,2
Porcentagem de grãos de pólen de aroeira (%)

Fig. 13. Compostos bioativos presentes nas amostras de pólen apícola com diferentes porcentagens de grãos de pólen de *Astronium urundeuva* em relação à sazonalidade (A-D). (E) Atividade antioxidante pelo método do complexo de fosfomolibdênio (PCM). Barras verticais representam o desvio padrão das médias, letras diferentes indicam diferença significativa pelo teste de Tukey ($P<0,05$).

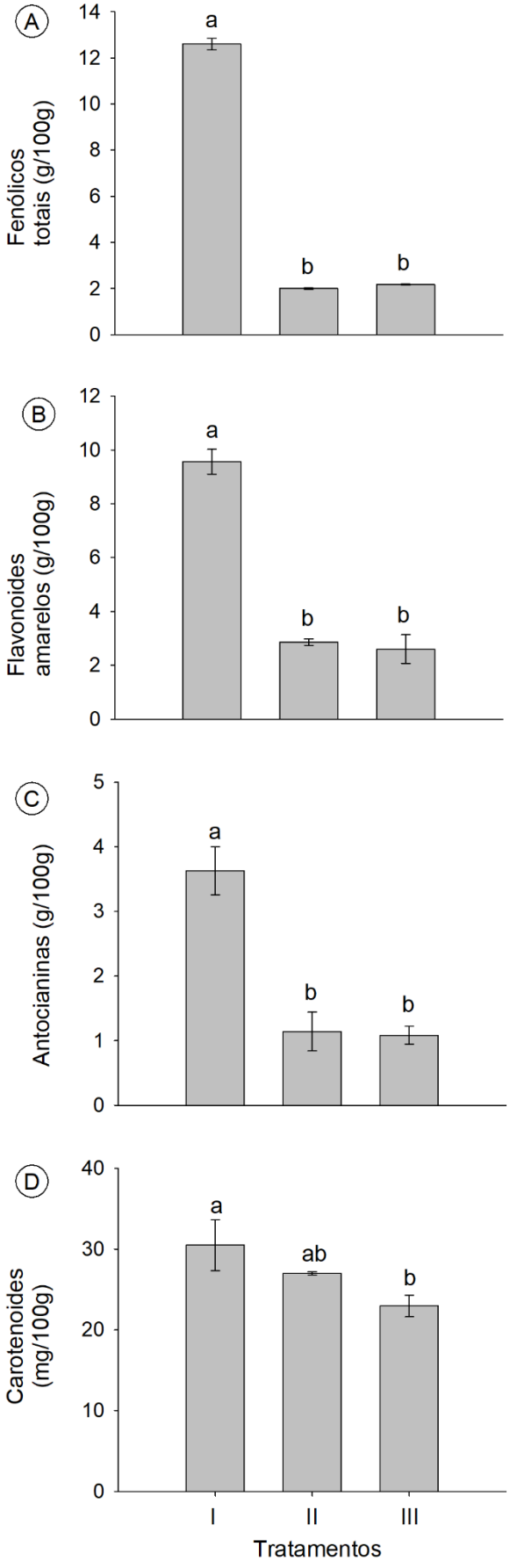
A
Fenólicos totais (g/100g)
14
12
10
8
6
4
2
0
a
b
b
B
Flavonoides amarelos (g/100g)
12
10
8
6
4
2
0
a
b
b
C
Antocianinas (g/100g)
5
4
3
2
1
0
a
b
b
D
Carotenoides (mg/100g)
40
30
20
10
0
a
ab
b
I
II
III
Tratamentos

Fig. 14. Compostos bioativos presente na amostra de pólen apícola com 84,2% de grãos de pólen de *Astronium urundeuva* em relação ao armazenamento (A-D). Tratamentos: I) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 30 dias; II) pólen apícola *in natura* mantido sob refrigeração, a -21 ºC, por 240 dias; e III) pólen apícola desidratado e armazenado sob abrigo da luz, por 90 dias.

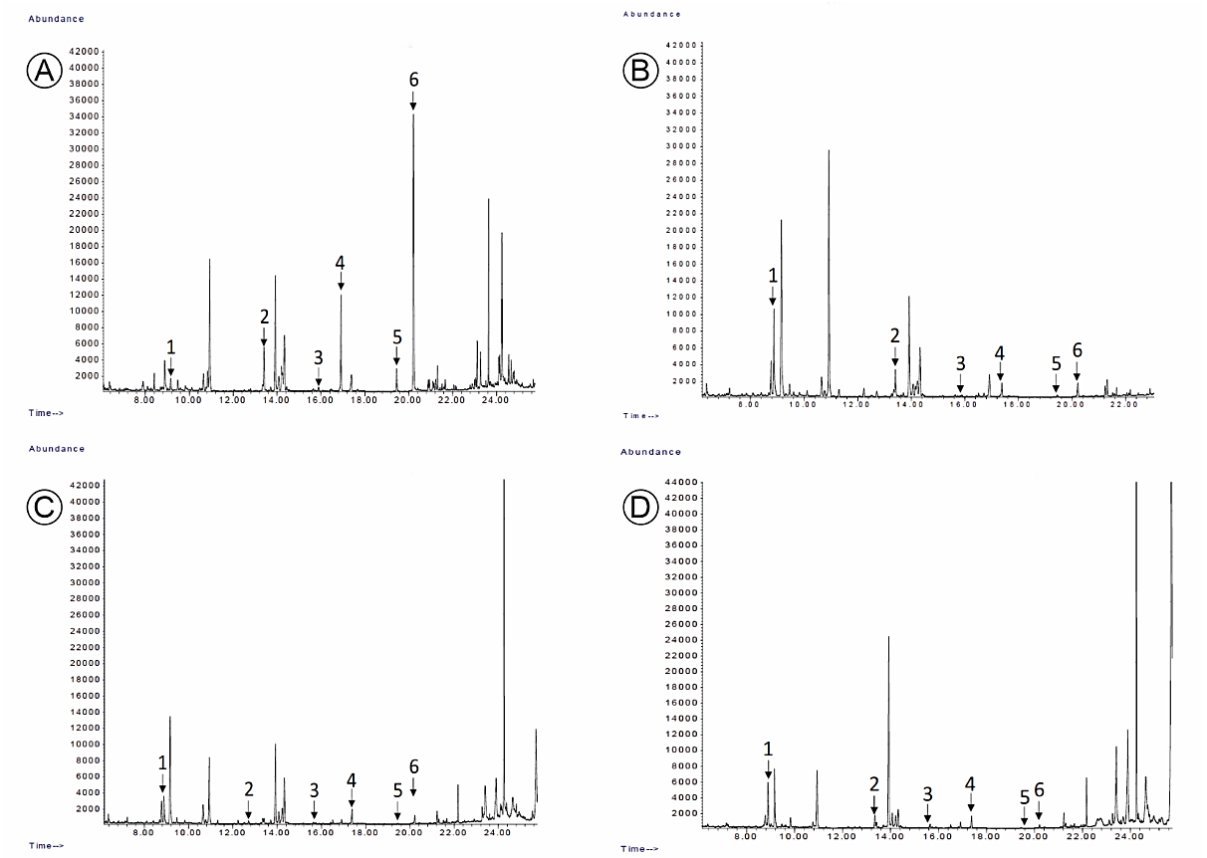

Fig. 15. Cromatogramas relativos às análises de ácidos fenólicos de quatro amostras de pólen apícola com diferentes porcentagens de grãos de pólen de *Astronium urundeuva* e diferentes formas de armazenamento. (A) março - 0% congelado, (B) maio - 79,8% congelado, (C) junho - 84,2% congelado (D) junho – 84,2% desidratado (D). Ácidos fenólicos: 1) salicílico, 2) vanílico, 3) siríngico, 4) gálico, 5) ferúlico e 6) cafeico.

8. ANEXO 1

Journal of Agricultural and Food Chemistry

Manuscript Submission Requirements Checklist

Manuscripts and revised manuscripts must be submitted via the ACS Paragon Plus Web site (acsparagonplus.acs.org). E-mailed submissions and hardcopy submissions will not be processed. An overview of and complete instructions for the Web submission process are available at the ACS Paragon Plus website.

When submitting, please be aware of the following requirements:

All manuscripts must be accompanied by a written statement on the manuscript's significance (not a summary of the abstract), addressing the following three bullet points: (1) statement of the problem addressed and originality of the approach, (2) contribution of the work to create new knowledge in the field, and (3) relevance of the work to advance research and impact to the field of agricultural and food chemistry, including the specific role of molecular research in the study. This 3-point statement should be uploaded as an informal letter using the manuscript file designation "Supporting information for review only".

All co-authors listed on the title page of the manuscript must be entered into the ACS Paragon Plus System at step 2 in the manuscript submission process. Only one corresponding author is allowed for each manuscript in Paragon Plus. Additional corresponding authors may be designated on the manuscript title page. Use of the phrase "all authors contributed equally" is discouraged. Instead, statements about author contributions should identify the specific aspect of the author's contribution.

The manuscript abstract and text must appear in a single, double-spaced column; lines in the abstract and text must be numbered consecutively from beginning to end in a separate column

at the left. There is no separate conclusion section to be used; conclusions should be incorporated into the results and discussion section. All of the manuscript text (including title page, abstract, all sections of the body of the paper, figure captions, scheme or chart titles and footnotes, and references) and tabular material should be in one file, with the complete text first followed by the tabular material.

To ensure that a submitted manuscript meets sufficient interest of the readership of the journal, it is expected that articles recently published on the respective topic in the *Journal of Agricultural and Food Chemistry* and other similar journals in the field are cited to a reasonable extent. In general, references must be numbered in the order in which they appear in the text.

The author's preference for manuscript category is indicated during the submission process. However, the final decision on the category under which the manuscript will be listed lies with the Editor.

The system requires authors to supply the names, e-mail addresses, and affiliations of at least four recommended reviewers. The recommended reviewers should be experts in the subject matter of the manuscript and not be anyone who is or has been a former adviser/advisee,colleague in the same institution, research collaborator, and/or co-author of papers and patents or in any other way has a conflict of interest.

If the manuscript is one of a series of companion manuscripts that will be published sequentially, please describe the planned series in the cover letter, mentioning previously published parts and giving an estimate of when subsequent parts will be submitted.

Complete instructions for manuscript preparation are updated frequently and are available at the *Journal*'s website. Please conform to these instructions when submitting manuscripts.

Authors whose manuscripts are published in *Journal of Agricultural and Food Chemistry* will be expected to review manuscripts submitted by other researchers from time to time.

Scope Of The Journal

The *Journal of Agricultural and Food Chemistry* considers high-quality, original research representing complete studies and scientific advances dealing with the innovative application of chemistry, biochemistry and biological sciences to increase the molecular understanding of product attributes, processes, technologies, and health aspects encompassing the agricultural-food-nutrition continuum.

Manuscripts are expected to involve chemistry, biochemistry and/or molecular biology as the fundamental component and can be combined with novel aspects of process engineering and food technology, authenticity and origin aspects of food, or the biological evaluation of agricultural systems including plant-plant, plant-fungal and plant-insect interactions, and/or food systems. The latter may include microbial, nutritional, physiological, sensory, or toxicological properties, and data must accompany sufficient discussion to demonstrate their relevance to food and nutrition.

Research considered for publication should be of general interest to the scientific community and/or the public, its potential impact should be significant and the technical quality is expected to conform to the highest standards of chemical research.

Current specific categories are as follows:

Agricultural and Environmental Chemistry

to advance molecular knowledge (e.g., crop protection chemistry, nanotechnology, natural toxins, fate and biotransformation of residues), elucidate mechanisms of action of

agrochemicals, understand mechanisms of plant-plant, plant-fungal and plant-insect interactions including the action of bioactive constituents in desirable plants on control of pests that threaten them, and promote innovative solutions for increased agricultural productivity, a sustainable supply of food and fiber, and protection of public health and the environment including water quality/treatment, agricultural waste, and energy- related issues.

Bioactive Constituents, Metabolites, and Functions

to increase knowledge of chemical structures of bioactive constituents, phytonutrients, and nutraceuticals in foods, their human and animal metabolism, and their mechanisms of biological function to affect human health status, including various aspects of molecular nutrition such as nutritional biochemistry, nutrigenomics and metabolomics. Studies on traditional medicines and herbal remedies are outside the scope of this category. It is mandatory that manuscripts reporting on biological properties of crude extracts include detailed information on the chemical composition of the extracts causing the described properties.

Manuscripts can encompass cell-based or other in vitro assays, animal models, clinical human trails, or a combination thereof as the fundamental component, however, test systems applied must be appropriate and the analytical method used should allow the quantitation of time and dose response effects. It is understood that discussion on nutritional relevance and conclusions on human health aspects are carefully formulated considering the experimental design used (appropriate cell test lines, significance of test/trail, relevant dose levels) and the data obtained.

Biofuels and Biobased Materials

to advance knowledge of chemistry, biology, and processing of biobased products and biofuels including all the related areas of biodiesel, bioethanol, biogas, biotransformations and bioprocesses (plants, algae etc.), waste utilization, biorefinery and bioresource technologies

associated with conversion or production of biobased materials, and sustainability, and environmental emissions and effects associated with these processes.

Biotechnology and Biological Transformations

to foster technological advances in plant/agricultural biotechnology (e.g., crop improvement, nutraceuticals, bioenergy, transgenic plants, phytoremediation), microbial and insect biotechnology (metabolic engineering and synthetic/systems biology of bacteria, fungi, insects, yeasts and algae in the context of fermentation/bioproduction, biocatalysis, bioremediation, biodegradation), food and flavor biotechnology (biotransformations/microbiology and metabolic aspects of food/beverage systems), and protein and enzyme technology (recombinant proteins/enzymes, cell-free protein expression systems, and biocatalysis using immobilized enzymes).

Chemistry and Biology of Odor and Taste

to advance molecular knowledge of the instrumental analysis (e.g. bioelectronics sensors), chemical structures, and formation pathways of aroma and taste molecules of plant and animal derived foods and beverages, the chemosensory receptors mediating flavor object recognition (e.g. *in vitro* cell assays), human *in vivo* psychophysics (incl. multisensory integration), and neurological processing of flavor stimuli (imaging technologies).

Food and Beverage Chemistry/Biochemistry

to deepen the fundamental understanding of chemical structures, structural modifications, interactions, and (bio)chemical transformations of minor and major components in foods and beverages, potentially in combination with novel aspects of process engineering, food

technology, nanotechnology, packaging and storing, authenticity and origin aspects of food, or the biological evaluation of food and beverage systems.

Food Safety and Toxicology

to advance our knowledge of detrimental health effects and the mechanisms of adverse physiological, or pathological changes induced by natural or synthetic chemicals occurring in the human environment with particular emphasis on foods, crop protection chemicals, contaminants and related chemicals (e.g., nanomaterials, biotechologically derived products), including agricultural safety and consumer product safety, and the design and action of chemically related processes that enhance food safety.

Functional Structure/Activity Relationships

to increase the knowledge on the relationship between chemical structure and biological (microorganisms, insects, animals, human) or technofunctional activity (e.g., Emulsifying, foaming, gelation) of agricultural and food components.

This category comprises (i) organic synthetic studies and/or structural biological studies (X-ray, NMR, etc.) of relevant ligands and targets with the aim of investigating molecular recognition processes in the action of biologically active compounds, (ii) molecular biological studies (e.g., site-directed mutagenesis) of macromolecular targets that lead to an improved understanding of molecular recognition, and (iii) computational studies that analyze the SAR of compounds of interest and lead to experimental studies or analysis of other available chemical and/or biological data that substantially advance the knowledge in agricultural and food chemistry.

Routine extensions of existing series that do not add significantly to a basic understanding of the structure-activity relationship (SAR) of the series or do not utilize novel chemical/biological approaches will normally not be considered for publication.

New Analytical Methods

to expand the repertoire of analytical methods in agriculture and food research by new analytical method development using chemical, physical, and biological principles. Manuscripts dealing with existing analytical methods should offer a significant, original application of the method or a major improvement going far beyond state-of-the-art.

For manuscripts describing the application of an existing method, even when modified, the category selected should be driven by the application (e.g., Agricultural and Environmental Chemistry, Bioactive Constituents, Metabolites, and Functions, etc.).

Omics Technologies Applied to Agriculture and Food

to promote a more integrative understanding of complex systems in agriculture, food, and nutrition by the application of metabolomics, proteomics, and transcriptomics/genomics technologies combined with, but not limited to, bioinformatics and computational biology.

It is mandatory that manuscripts in this category go beyond a sheer holistic fingerprinting of samples, metabolic changes need to be identified on a molecular level and validated by means of targeted analysis.

These categories are periodically reviewed and may be changed.

Manuscript Types

RESEARCH ARTICLES must report original research that is expected to have a definable impact on the advancement of science and technology, incorporating a significant component of innovative chemistry and/or molecular biology. Novel experimental results, theoretical treatments, interpretations of data, and absence of prior publications on the same/similar topics will document originality. Fragmentation of work into an incremental series of manuscripts is not acceptable.

REVIEW ARTICLES will be considered that comprehensively summarize information in a field in which the literature is scattered and/or treat published data or other information so as to provide a new approach or stimulate further research. Authors considering the preparation of a review may contact the Editor with any questions.

PERSPECTIVES, as opposed to a comprehensive Review Article, are expected to be concise discussions of a particular field to help readers keep abreast of the advances and trends in agricultural and food chemistry outside their own area of expertise. Therefore, Perspectives are written in a manner understandable to scientists working in any area under the broader umbrella of agricultural and food chemistry. Following an abstract of no more than 100 words, the text of the Perspective should not exceed 12 double-spaced manuscript pages in length, exclusive of tables, figures, photographs, and references. Up to four tables, figures, or photographs, total, may be included. References should be limited to no more than 30 in total.

VIEWPOINTS are short opinion-style manuscripts that provide authors with a venue to comment on an issue of pressing importance to the JAFC readership community. Viewpoints are not peer-reviewed but are subject to editorial approval. JAFC welcomes Viewpoints of a scientific nature; no Viewpoints of an exclusively political nature will be considered for publication. Successful Viewpoints clearly articulate a research need to the reader and avoid

summarizing a particular research area or study. A limit of 1000 words + author affiliations + 5 references + 1 single-frame figure with a 50 word caption OR a 350 word table will be strictly enforced; submissions exceeding this maximum will not be considered.

COMMENTS related to published papers will be considered from readers if the correspondence is received within six months of the date of publication of the original paper; the authors of the original paper will be given the opportunity to reply to such comments within two months, if they so desire.

Both comments and replies should not exceed 1000 words each, including citations, and will be published consecutively in the same issue of the *Journal* after peer review. For examples, see *J. Agric. Food Chem.*, 2015, 63, 5305–5306 (DOI: 10.1021/jf506172q) and J. Agric. Food Chem., 2015, 63, 5307–5307 (DOI: 10.1021/acs.jafc.5b01143).

SYMPOSIA OR TOPICAL COLLECTIONS comprise a series of manuscripts reporting or synthesizing original research that are presented in a symposium or otherwise clustered around a single topic. Prospective organizers should contact the Editor well in advance to determine whether the subject matter conforms to the *Journal*'s goals, criteria, and available space and to obtain specific instructions for submission of the manuscripts. Each manuscript will be subject to the normal peer-review process. For an example, see *J. Agric. Food Chem.*, 2015, 63, 5837–5840 (DOI: 10.1021/acs.jafc.5b00324) and J. Agric. Food Chem., 2015, 63, 5099–5099.(DOI: 10.1021/acs.jafc.5b00159).

ACS Publishing Center

While this document will provide basic information on how to prepare and submit the manuscript as well as other critical information about publishing, we also encourage authors to

visit the ACS Publishing Center for additional information on everything that is needed to prepare (and review) manuscripts for ACS journals and partner journals, such as

Mastering the Art of Scientific Publication, which shares editor tips about a variety of topics including making your paper scientifically effective, preparing excellent graphics, and writing cover letters.

Resources on how to prepare and submit a manuscript to ACS Paragon Plus, ACS Publications' manuscript submission and peer review environment, including details on selecting the applicable Journal Publishing Agreement.

Sharing your research with the public through the ACS Publications open access program.

ACS Reviewer Lab, a free online course covering best practices for peer review and related ethical considerations.

ACS Author Lab, a free online course that empowers authors to prepare and submit strong manuscripts, avoiding errors that could lead to delays in the publication process.

ACS Inclusivity Style Guide, a guide that helps researchers communicate in ways that recognize and respect diversity in all its forms.

Manuscript Preparation

Submit with Fast Format

All ACS journals and partner journals have simplified their formatting requirements in favor of a streamlined and standardized format for an initial manuscript submission. Read more about the requirements and the benefits these serves authors and reviewers here.

Manuscripts submitted for initial consideration must adhere to these standards:

Submissions must be complete with clearly identified standard sections used to report original research, free of annotations or highlights, and include all numbered and labeled components.

Figures, charts, tables, schemes, and equations should be embedded in the text at the point of relevance. Separate graphics can be supplied later at revision, if necessary.

When required by a journal's structure or length limitations, manuscript templates should be used.

References can be provided in any style, but they must be complete, including titles. For information about the required components of different reference types, please refer to the ACS Style Quick Guide.

Supporting Information must be submitted as a separate file(s).

Document Templates and Format

The *Journal of Agricultural and Food Chemistry* does not require the use of any document templates. General information on the preparation of manuscripts may be found in the ACS Guide to Scholarly Communication.

Acceptable Software, File Designations, and TeX/LaTeX

See the list of Acceptable Software and appropriate File Designations to be sure your file types are compatible with ACS Paragon Plus. Information for manuscripts generated from TeX/LaTeX is also available.

Cover Letter

A cover letter must accompany every manuscript submission. During the submission process, you may type it or paste it into the submission system, or you may attach it as a file.

Manuscript Text Components

MANUSCRIPT FORMAT

The Journal has a 20 typed page limit, not including references, tables, and figures. Authors must request approval from the Editor-in-Chief to submit manuscripts exceeding 20 typed pages.

The various sections of the manuscript should be assembled in the following sequence:

Title and authorship (single page)

Abstract and keywords (single page)

Introduction

Materials and Methods (including Safety information)

Results/Discussion

Abbreviations Used

Acknowledgment

Supporting Information description

References

Graphic for table of contents

TITLE, AUTHORSHIP, AND KEYWORDS

Title. The title should be specific, informative, and concise. Keywords in the title assist in effective literature retrieval. If a plant is referred to in the title or elsewhere in the text by its common or trivial name, it should be identified by its scientific name in parentheses

immediately following its first occurrence. This term should also be provided as one of the keywords. If trade names are mentioned, give generic names in parentheses.

Authorship. Be consistent in authorship designation on the manuscript and on all correspondence. First name, middle initial, and last name are generally adequate for correct identification, but omit titles. Give the complete mailing address of all institutions where work was conducted and identify the affiliation of each author. If the current address of an author is different, include it in a footnote on the title page. The name of the author to whom inquiries about the paper should be addressed must be marked with an asterisk; provide the telephone number and e-mail address of this correspondent.

Many Funders and Institutions require that institutional affiliations are identified for all authors listed in the work being submitted. ACS facilitates this requirement by collecting institution information during manuscript submission under Step 2: Authors and Affiliations in ACS Paragon Plus.

Keywords. Provide significant keywords to aid the reader in literature retrieval. Please consider the use of words different from those in the title to expand discoverability of the article. The keywords are published immediately before the text, following the abstract.

ABSTRACT

Authors' abstracts are used directly for *Chemical Abstracts*. The abstract should be a clear, concise (100– 150 words), one-paragraph summary, informative rather than descriptive, giving scope and purpose, experimental approach, significant results, and major conclusions. Write for literature searchers as well as journal readers.

INTRODUCTION

Discuss relationships of the study to previously published work, but do not reiterate or attempt to provide a complete literature survey. Use of *Chemical Abstracts/Scifinder* and other appropriate databases is encouraged to ensure that important prior publications or patents are cited and that the manuscript does not duplicate previously published work. The purpose or reason for the research being reported, and its significance, originality, or contribution to new knowledge in the field, should be clearly and concisely stated. Current findings should not be included or summarized in this section.

MATERIALS AND METHODS

Authors must emphasize any unexpected, new, and/or significant hazards or risks associated with the reported work. This information should be in the experimental details section of the full article or communication.

Apparatus, reagents, and biological materials used in the study should be incorporated into a general section. List devices of a specialized nature or instruments that may vary in performance, such that the model used may affect the quality of the data obtained (e.g., spectroscopic resolution).

List and describe preparation of special reagents only. Reagents normally found in the laboratory and preparations described in standard handbooks or texts should not be listed.

Specify the source, vendor [city and state (or city and country if non-U.S.)], and availability of special equipment, reagents, kits, etc. Do not include catalog numbers.

Biological materials should be identified by scientific name (genus, species, authority, and family) and cultivar, if appropriate, together with the site from which the samples were obtained. Specimens obtained from a natural habitat should be preserved by deposit of samples

in an herbarium for plants or in a culture collection for microorganisms, with a corresponding collection or strain number listed.

Manuscripts describing studies in which live animals or human subjects are used must include a statement that such experiments were performed in compliance with the appropriate laws and institutional guidelines and also name the institutional committee that approved the experiments. Authors are encouraged to note the approval code or number or give the name of the approving office or official. (See Reporting Specific Data: Animal or Human Studies.) Manuscripts reporting data from inhumane treatment of experimental animals will be rejected.

Specific experimental methods should be sufficiently detailed for others to repeat the experiments unequivocally. Omit details of procedures that are common knowledge to those in the field. Brief highlights of published procedures may be included, but details must be left to the References, and verbatim repeat of previously published methods, even if done by the authors, will not be permitted unless a quotation from a published work is included, and placed in quotation marks, with the reference to the source included at the end of the quotation. Describe pertinent and critical factors involved in reactions so the method can be reproduced, but avoid excessive description. For information on the reporting of certain types of data see Reporting Specific Data.

RESULTS AND DISCUSSION

Results and discussion may be presented in separate sections or combined into a single section, whichever format conveys the results in the most lucid fashion without redundancy. Be complete but concise in discussing findings, comparing results with previous work and proposing explanations for the results observed.

All data must be accompanied by appropriate statistical analyses, including complete information on sampling, replication, and how the statistical method employed was chosen.

Avoid comparisons or contrasts that are not pertinent, and avoid speculation unsupported by the data obtained.

A separate summary or conclusion section is not to be used; any concluding statements are to be incorporated under Results and Discussion.

ABBREVIATIONS AND NOMENCLATURE

Standard abbreviations, without periods, should be used throughout the manuscript.

Refer to *The ACS Style Guide* for the preferred forms of commonly used abbreviations. Specialized abbreviations may be used provided they are placed in parentheses after the word(s) for which they are to substitute at first point of use and are again defined in this section. Avoid trivial names and "code" abbreviations (e.g., NAR for naringenin) unless such codes are in common usage (e.g., MTBE for methyl *tert*-butyl ether).

If trade names are used, define at point of first use. If nomenclature is specialized, include a "Nomenclature" section at the end of the paper, giving definitions and dimensions for all terms. Use SI units insofar as possible. Refer to *The ACS Style Guide* for lists of SI units and a discussion of their use.

Write all equations and formulas clearly and number equations consecutively. Place superscripts and subscripts accurately; avoid superscripts that may be confused with exponents. Identify typed letters and numbers that might be misinterpreted, such as "oh" for zero or "ell" for one. Chemistry numbering requiring primes should be identified as such (i.e., 3,3´-dihydroxy-), not by an apostrophe (e.g., 3,3'- dihydroxy-).

It is the authors' responsibility to provide correct nomenclature. Structures should be included for uncommon chemicals, particularly when the systematic or common name is too complex or unclear to readily denote the structure. Such structures should be included as a figure or table. All nomenclature must be consistent and unambiguous and should conform to current American usage. Insofar as possible, authors should use systematic names similar to those used by Chemical Abstracts Service, the International Union of Pure and Applied Chemistry, and the International Union of Biochemistry and Molecular Biology. *Chemical Abstracts* (CA) nomenclature rules are described in Appendix IV of the *Chemical Abstracts Index Guide*. For CA nomenclature advice, consult the Manager of Nomenclature Services, Chemical Abstracts Service, P.O. Box 3012, Columbus, OH 43210-0012. A name generation service is available for a fee through CAS Client Services, 2540 Olentangy River Road, P.O. Box 3343, Columbus, OH 43210-0334 [telephone (614) 447-3870; fax (614) 447-3747; e-mail answers@cas.org].

ACKNOWLEDGMENT

Include essential credits but hold to an absolute minimum. Omit academic and social titles. Meeting presentation data and acknowledgment of financial support of the work should not be included here; give these instead in a note following the References. It is the responsibility of the corresponding author to notify individuals named in the Acknowledgment prior to submission.

FUNDING SOURCES

Authors are required to report ALL funding sources and grant/award numbers relevant to the manuscript. Enter all sources of funding for ALL authors relevant to the manuscript BOTH in the Open Funder Registry tool in ACS Paragon Plus and in the manuscript to meet this

requirement. See http://pubs.acs.org/page/4authors/funder_options.html for complete instructions.

Funding should be acknowledged in a separate statement (not in the Acknowledgment paragraph).

REFERENCES

Consult *The ACS Style Guide* and current issues of the *Journal* for examples of reference format.

Authors should cite all prior published work directly pertinent to the manuscript. To demonstrate that the submitted manuscript meets sufficient interest of the readership of the journal, it is expected that articles recently published on the respective topic in the *Journal of Agricultural and Food Chemistry* and other similar journals in the field are cited to a reasonable extent. As a general guideline, authors should attempt to limit the literature cited to approximately 50 or fewer citations (except for Review manuscripts).

Authors are responsible for the accuracy of their references. References taken from a review or other secondary source should be checked for accuracy with the primary source.

References should be listed on a separate page and numbered in the order in which they are cited in the text.

References should be cited in the text by superscript numbers, for example, *1*,*2*–*5*, etc.

Give complete information, using the last name and initials of the author, patentee, or equivalent; do not use “Anonymous”.

Follow *Chemical Abstracts Service Source Index* for abbreviations of journal titles. Because subscribers to the Web edition of the *Journal* are now able to click on the "Chemport" or other tag following each reference to retrieve the corresponding abstract from various Web resources, reference accuracy is critical.

Typical references follow the styles given below.

For journals:

Brown, J.; Jones, M.; Green, D. Article title. *J. Agric. Food Chem.* 1980, *28*, 1–4. (Issue number must be used if each issue of the periodical begins with page)

For books:

Smith, L; Caldwell, A. Chapter title. In *Book Title*, edition no.; Keys, F., Park, G., Eds.; Publisher: City, State (or Country if non-U.S.), Year; Vol. no., pp.

For Web pages:

Black, A.; White, B. Page title. URL (http://...) (most recent access date).

Papers should not depend for their usefulness on unpublished material, and excessive reference to material "in press" is discouraged. Reference to the authors' own unpublished work is permitted if the subject is of secondary importance to the manuscript in question, but any unpublished results of central importance must be described in sufficient detail within the manuscript. If pertinent references are "in press" or unpublished for any reason, furnish copies to enable reviewers to evaluate the manuscript. An electronic copy of these materials should be uploaded according to the directions for review-only Supporting Information. "In press" references should include the Digital Object Identifier (DOI) assigned by the potential publisher.

TABLES AND ARTWORK

Tables and figures should be carefully designed to maximize presentation and comprehension of the experimental data with superfluous information excluded. Tables must be self-contained, that is, understandable without reading the text of the article. This includes using footnotes to explain sample names, units, and other relevant information. Useful information not directly relevant to the discussion may be included under Supporting Information.

Tables. Tables may be created using a word processor's text mode or table format feature. The table format feature is preferred. Ensure each data entry is in its own table cell. Lower case should be used for all table entries unless a capital letter is required. If the text mode is used, separate columns with a single tab and use a line feed (enter) at the end of each row.

Tables should be numbered consecutively with Arabic numerals and should be grouped after the figure captions. Footnotes in tables should be given letter designations and be cited in the table by italic superscript letters. The sequence of letters should proceed by row rather than by column. Each table should be provided with a descriptive heading, which, together with the individual column headings, should make the table, as nearly as possible, self-explanatory. In setting up tabulations, authors are requested to keep in mind the type area of the journal page (17.8 × 25.4 cm), and the column width (8.5 cm), and to make tables conform to the limitations of these dimensions. Arrangements that leave many columns partially filled or that contain much blank space should be avoided. Conversely, arrangements that include >20 columns should be broken into two tables if possible. If *significance of values* is to be indicated, use a lower case letter, on line, one space after the value.

Figures and Artwork. The preferred submission procedure is to embed graphic files in a Word document. It may help to print the manuscript on a laser printer to ensure all artwork is clear

and legible. Artwork should be sequentially numbered using Arabic numbers. Schemes and charts may have titles and footnotes; figures should have captions. Insert the captions following the References and the graphics after the Tables.

Additional acceptable file formats are TIFF, PDF, EPS (vector artwork), or CDX (ChemDraw file). If submitting individual graphic files in addition to their being embedded in a Word document, ensure the files are named according to graphic function (i.e., Scheme 1, Figure 2, Chart 3), not the scientific name.

Labeling of all figure parts should be present, and the parts should be assembled into a single graphic. For EPX files, ensure that all fonts are converted to outlines or embedded in the graphic file. The document setting should be in RGB mode. Note: Although EPS files are accepted, the vector-based graphics will be rasterized for production. Please see below for TIFF file production resolutions.

TIFF files (either embedded in a Word document or submitted as individual files) should have the following resolution requirements: black and white line art, 1200 dpi; grayscale art (a monochromatic image containing shades of gray), 600 dpi; color art (RGB color mode), 300 dpi.

The RGB and resolution requirements are essential for producing high-quality graphics within the published paper. Graphics submitted in CMYK or at lower resolution may be used; however, the colors may not be consistent. Graphics of poor quality may not be able to be improved.

Most graphic programs provide an option for changing the resolution when images are saved. Best practice is to save the graphic file at the final resolution and size using the program used to create the graphic.

For bar charts, bars with hatching patterns generally reproduce well. Bars that range in shading from light to dark gray to black can usually be reproduced successfully, although we do not recommend any more than two shades of gray. A legend needs to be included within the figure itself rather than the patterns or shades included in the caption.

For manuscripts containing gel patterns, use of a high-resolution digital scanner is recommended. Only high-quality original, unaltered digital reproductions will allow reviewers to correctly verify the experimental results. For an example of gel patterns see *J. Agric. Food Chem.*, 2012, 60 (18), 4492–4499 (DOI: 10.1021/jf300563n).

Only readable and accurately represented images are acceptable; the Editors reserve the option to reject images that do not satisfactorily support points made in the manuscript or that are not of satisfactory quality for publication.

The quality of the illustrations published in the *Journal* largely depends on the quality of the originals provided. Figures cannot be modified or enhanced by the journal production staff. Contrast is important. Each figure or photograph should be properly labeled.

Structural Formulas. Structural formulas should be included for all new chemicals and for existing chemicals for which chemical nomenclature and/or trivial names do not convey the structure adequately. Structural formulas are valuable in expressing concisely the precise nature of the compounds under discussion and revealing the essence of the subject to readers unfamiliar with the topic, without their necessary recourse to reference materials. The use of chemical names without accompanying structures may cause readers to overlook the significance of the paper.

Structures should be produced with the use of a drawing program such as ChemDraw. Structure drawing requirements (preset in the ACS Stylesheet in ChemDraw) are as follows:

As drawing settings, select:

chain angle, 120º

bond spacing, 18% of width

fixed length, 14.4 pt (0.508 cm, 0.2 in.)

bold width, 2.0 pt (0.071 cm, 0.0278 in.)

line width, 0.6 pt (0.021 cm, 0.0084 in.)

margin width, 1.6 pt (0.056 cm, 0.0222 in.)

hash spacing, 2.5 pt (0.088 cm, 0.0347 in.)

As text settings, select:

font, Arial/Helvetica

size, 10 pt

Under the preferences, choose:

units, points

tolerances, 5 pixels

Under page setup, choose:

paper, US Letter

scale, 100%

Using the ChemDraw ruler or appropriate margin settings, create structure blocks, schemes, and equations having maximum widths of 11.3 cm (one-column format) or 23.6 cm (two-

column format). Note: if the foregoing preferences are selected as cm values, the ChemDraw ruler is calibrated in cm. Also note that a standard sheet of paper is only 21.6 cm wide, so all graphics submitted in two-column format must be prepared and printed in landscape mode.

Use boldface type for compound numbers but not for atom labels or captions.

Authors using other drawing packages should, as far as possible, modify their program's parameters to reflect the above guidelines.

REVISIONS AND RESUBMISSIONS

For all revisions:

Clearly identify the manuscript as a revision; reference the manuscript number.

Include an itemized list of changes, with a response to each comment made by the Editor and by each reviewer.

Be aware that the manuscript may be sent for additional review, to the same or additional reviewers, at the discretion of the Editor.

For all resubmissions:

Clearly identify all resubmissions; reference the previous manuscript number.

Include an itemized list of changes, including a response to each comment made by the Editor and by each reviewer.

Supporting Information

This information is provided to the reviewers during the peer-review process (for Review Only) and is available to readers of the published work (for Publication). Supporting Information must

be submitted at the same time as the manuscript. See the list of Acceptable Software by File Designation and confirm that your Supporting Information is viewable.

If the manuscript is accompanied by any supporting information files for publication, these files will be made available free of charge to readers. A brief, nonsentence description of the actual contents of each file, including the file type extension, is required. This description should be labeled Supporting Information and should appear before the Acknowledgement and Reference sections. Examples of sufficient and insufficient descriptions are as follows:

Examples of sufficient descriptions: "Supporting Information: ^{1}H NMR spectra for all compounds (PDF)" or "Additional experimental details, materials, and methods, including photographs of experimental setup (DOC)".

Examples of insufficient descriptions: "Supporting Information: Figures S1-S3" or "Additional figures as mentioned in the text".

When including supporting information for review only, include copies of references that are unpublished or in-press. These files are available only to editors and reviewers.

Research Data Policy

All ACS journals strongly encourage authors to make the research data underlying their articles publicly available at the time of publication.

Research data is defined as materials and information used in the experiments that enable the validation of the conclusions drawn in the article, including primary data produced by the authors for the study being reported, secondary data reused or analyzed by the authors for the study, and any other materials necessary to reproduce or replicate the results.

The ACS Research Data Policy provides additional information on Data Availability Statements, Data Citation, and Data Repositories.

Data Requirements

Bioactivity. Manuscripts reporting on key bioactive constituents in agricultural products, foods, and beverages and on the mechanisms of how these compounds promote health in living organisms, including humans, livestock and domestic animals are expected to follow a cutting edge chemical, biochemical, and/or molecular biological approach. For the identification of a bioactive agricultural/food compound, an activity-guided fractionation approach should be followed, with generally accepted criteria for complete chemical characterization of the bioactive compound's molecular structure using state-of-the-art analytical tools (TOF-MS, 1D/2D-NMR etc.).

Manuscripts can encompass cell-based or other in vitro assays, animal models, human intervention studies, clinical trials, or a combination thereof as the fundamental component, however, target compounds need to be tested at relevant dose levels, test systems applied must be validated, should allow the quantitation of time and dose response effects, and need to be appropriate for in vivo conditions. In order to demonstrate bioefficacy as an overall aim of the 'Bioactives Research' theme, the bioavailability of the target food constituent has to be substantial and may, in some cases, not be sufficient to exert the desired effect after dietary intake. Therefore, the discussion on nutritional relevance and conclusions on human health aspects need to be carefully formulated considering the experimental design used (appropriate cell-based or other in vitro assays, animal models, clinical human trials, significance of test/trial, relevant dose levels etc.), the robustness of the data set obtained, and addressing the underlying mechanism of action.

It is mandatory that manuscripts reporting on biological properties of individual constituents include information on the purity of the test components and on how it has been determined (e.g., ^{1}H NMR, GC- FID, HPLC-ELSD). Similarly, investigations performed with crude extracts need to present detailed information on the chemical composition of the extracts responsible for the described properties. This means that key representatives of the chemical class investigated (e.g., polyphenols, terpenoids, alkaloids, peptides) should be quantitatively fingerprinted.

Gas Chromatographic Methods. For manuscripts in which gas chromatographic methods are used, see "Reporting of Gas Chromatographic Methods", by Morton Beroza and Irwin Hornstein [*J. Agric. Food Chem*. 1973, *21*, 7A (located at the back of the January 1973 issue or as a link from the *Journal*'s Author Information page)]. Consult recent issues for examples of GC, LC, and other instrument parameter descriptions.

Spectroscopic Data. This is a guide only; in certain cases different methods of data presentation may be more suitable. Authors are encouraged to consult examples of data presentation published in recent issues of the *Journal* for appropriate style and format. Complete NMR, mass spectrometric, or other spectral data will be published only if novel or necessary to substantiate points made under the Results or Discussion sections. Such presentations take up valuable space, and essentially the same information can frequently be put into a much more compact form by simply listing the position and intensity of the maxima. It is usually not necessary to list all of the maxima in the spectra to provide an adequate description. Report the type of instrument used (e.g., in mass spectrometry, whether magnetic, quadrupole, time-of-flight, etc.) and also the type of cell, the solvent (if any), and the state of the sample (whether liquid, gas, solution, etc.).

Mass Spectra. List the molecular ion and about 10 of the major ions with their intensities in parentheses, or more preferably use the method outlined by H. S. Hertz, R. A. Hites, and K. Biemann (*Anal. Chem*. 1971, *43*, 681–691). This method involves dividing the spectrum into consecutive regions of 14 mass units starting at *m*/*z* 6 (i.e., 6–19, 20–33, 34–47, 48–61, etc.). The two most intense ions in each region are then listed. Intensities, relative to the most intense ion, the intensity of which is taken as 100, are shown in parentheses immediately following the *m*/*z* value; for example: hexanal, mass spectrum found (70 eV, two most intense ions each 14 mass units above *m*/*z* 34): 43 (86), 44 (100), 56 (86), 57 (65), 71 (28), 72 (33), 82 (18), 85 (5), 97 (2), 100 (2). If the molecular ion does not appear in this presentation, the author should indicate it separately.

Nuclear Magnetic Resonance (^{1}H NMR or ^{13}C NMR) Spectra. A document providing detailed information for the presentation of NMR data is now available through “Information for Authors and Reviewers” on the Journal’s home page.

The frequency, the solvent, and also the temperature (if other than ambient) used are first specified. The type of unit used (δ or τ) is then stated, followed by the position of the center of gravity of the sharp line, broad line, or spin–spin multiplet in these units. This is then followed by information in parentheses which (1) describes the type of splitting, that is, singlet as s, doublet as d, triplet as t, quadruplet as qd, multiplet as m; (2) gives the value of the number of protons the area represents; (3) gives the coupling constant *J*; and (4) gives the part of the molecule connected with the particular absorption with the protons involved underlined.

An example would be ^{1}H NMR for ethanol (60 MHz, CCl4): δ 1.22 (t, 3, *J* = 7 Hz, CH2<u>CH</u>3), 2.58 (s, 1, OH), 3.70 (qd, 2, *J* = 7 Hz, OC<u>H</u>2CH3).

Other Spectra. In general, list position and intensity of the maxima. In some cases it may be desirable to list points of inflection.

A brief explanation should be given for any abbreviations not in common use. Examples:

Reporting liquid chromatography (HPLC) and HPLC/MS: "Analysis of Polyphenolic Antioxidants from the Fruits of Three *Pouteria* Species by Selected Ion Monitoring Liquid Chromatography– Mass Spectrometry", by Jun Ma et al. *J. Agric. Food Chem.* 2004, *52*, 5873–5878.

Reporting data in detail, including UV shifts and IR spectra: "Characterization of Vegetable Oils: Detailed Compositional Fingerprints Derived from Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry", by Zhigang Wu et al. *J. Agric. Food Chem.* 2004, *52*, 5322–5328.

Novel Compound Characterization. For a discussion of the *Journal's* expectations for compound characterization, please read "Compound Identification: A *Journal of Agricultural and Food Chemistry* Perspective" by R. J. Molyneux and P. Schieberle. *J. Agric. Food Chem.* 2007, *55*, 4625–4629 (DOI: 10.1021/jf070242j). It is essential that novel compounds, either synthetic or isolated from natural sources, be characterized rigorously and unequivocally. Supporting data normally include physical form, melting point (if solid), UV/IR spectra if appropriate, ^{1}H and ^{13}C NMR, mass spectrometric data, and optical rotation (when compounds have chiral centers).

Examples:

Reporting X-ray data: "Racemic and Enantiopure Synthesis and Physicochemical Characterization of the Novel Taste Enhancer *N*-(1-Carboxyethyl)-6-

(hydroxymethyl)pyridinium- 3-ol Inner Salt", by Renaud Villard et al. *J. Agric. Food Chem*. 2004, *51*, 4040–4045 (DOI: 10.1021/jf034246+).

Reporting data in detail, including UV shifts: "Novel Flavonol Glycoside, 7-*O*-Methyl Mearnsitrin, from *Sageretia theezans* and Its Antioxidant Effect", by Shin-Kyo Chung et al. *J. Agric. Food Chem*. 2004, *52*, 4664–4668 (DOI: 10.1021/jf049526j).

Reporting data for previously known compounds: "Phenolic Constituents and Antioxidant Activity of *Wendita calysina* Leaves (Burrito), a Folk Paraguayan Tea", by Anna Lisa Piccinelli et al. *J. Agric. Food Chem*. 2004, *52*, 5863–5868 (DOI: 10.1021/jf040100e).

Flavor Constituents. Manuscripts reporting on flavor constituents should conform to the recommendations made by the International Organization of the Flavor Industry [for details, see the editorial in the October 1996 issue of *J. Agric. Food Chem*. (*44*, 2941–2941) (DOI: 10.1021/jf960654k)]. In brief, any identification of a flavoring substance must pass scrutiny of the latest forms of available analytical techniques. In practice, this means that any particular substance must have its identity confirmed by at least two methods, for example, comparison of chromatographic and spectrometric data (which may include GC, MS, IR, and NMR) with those of an authentic sample. If only one method has been applied (MS data alone or retention index or Kovats index alone), the identification shall be labeled "tentative". In addition, authors are encouraged to include at least semiquantitative data on the concentration of an identified component in the original source, for example, foodstuff or plant part. Ranges such as <1 μg/kg, 1–10 μg/kg, and 10–100 μg/kg are acceptable.

Flavor is evoked by smell (aroma) and taste. A good example showing the correct characterization of taste compounds is the study by Czepa and Hofmann (*J. Agric. Food Chem*. 2003, *51*, 3865–3873) (DOI: 10.1021/jf034085+). A good example for aroma

compound identification is (*J. Agric. Food Chem.*, 2000, 48 (6), pp 2430–2437) (DOI: 10.1021/jf991116l).

The use of reference compounds is a must, if data on sensory properties of single compounds are reported. Odor, which is perceived during sniffing of a food extract at a certain retention index, may be indicative of the presence of a given compound, but not conclusive unless substantiated by chromatographic and/or spectrometric data and comparison with an authentic reference compound.

Soil Classification. Soils used in research should be described down to the family level according to the soil classification scheme given in *Soil Taxonomy, A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd ed. (Agricultural Handbook 436; U.S. Government Printing Office: Washington, DC, 1999) (available on-line at http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/class/taxonomy). Also give series name if known.

This requirement is to allow comparison and extrapolation to other work giving similar soil classifications, as published in journals such as the *Journal of Soil Science, Soil Science Society of America Journal, Journal of Environmental Quality*, and *Geoderma*. If information is unavailable to classify the soils at the desired family level, classification should be described or estimated at least to the great group level in the same classification system.

Statistics. Manuscripts reporting analytical, biological activity, composition, and related data must include relevant statistical information to support discussion of differences or similarities in data sets. Refer to a standard statistics reference such as *Statistical Methods*, 8th ed.; Snedecor, G. W., Cochran, G., Eds.; University Press: Ames, IA, 1989.

Metabolomics. This category considers applications of metabolomics as related to research topics in agriculture, food, and nutrition, in particular metabolite-targeted analysis and progress in the development of analytical platforms for metabolomics approaches. A metabolome is the quantitative set of chemical compounds in a biological system, i.e., a food, at a given time. However, also metabonomics studies, focused on changes in a given metabolome, e.g., induced by environmental conditions or diseases, fall into this category.

Metabolic profiling and metabolomic fingerprinting correlated with multivariate or data-mining methods are acceptable, if presented in a targeted way. For additional information consult "Targeted Metabolomics: A New Section in the *Journal of Agricultural and Food Chemistry*" by J. N. Seiber, R. J. Molyneux, and P. Schieberle, *J. Agric. Food Chem.* 2013, (DOI: 10.1021/jf4046254).

Animal or Human Studies. Manuscripts describing studies in which the use of live animals or human subjects is involved must include under Materials and Methods a statement that such experiments were performed in compliance with the appropriate laws and institutional guidelines, and also name the institutional committee that approved the experiments. For experiments with human subjects, a statement that informed consent was obtained from each individual must be included and the consent forms made available to the *Journal* on request. Reviewers of manuscripts involving animal or human experiments will be asked to comment specifically on the appropriateness and conformity to regulations of such experiments. Authors are encouraged to note the approval code or number or give the name of the approving office of official.

Animal Subjects. The use of animals in a study should be employed only when there are no alternative methods for investigating the fundamental questions of the study. In such cases, it

is the ethical responsibility of all authors to ensure that the care of animals is of the highest possible order, that pain and/or distress is minimized, and that the numbers involved are strictly limited to those essential to fulfill the experimental design. In the United States the care and use of laboratory animals is regulated by the U.S. Department of Agriculture (USDA) under the Animal Welfare Act. Links to the regulations and other information are available at http://www.aphis.usda.gov/animal_welfare/links.shtml. It is recognized that researchers in other countries may be governed by different laws and regulations. In such cases, experiments should be designed to conform either to the above USDA regulations or to the International Guiding Principles for Biomedical Research Involving Animals (1985), available at http://www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm.

Human Subjects. The use of human subjects in experimental studies requires informed consent. Such consent requires that the subjects be informed completely not only about the procedures involved but also about the aims, design, and expected outcomes of the study. Consent must be obtained not only when subjects are involved directly in the study but also when samples (tissue, blood, plasma, etc.) are required for in vitro experiments. In the United States the protection of human research subjects is regulated by the U.S. Department of Health and Human Services (HHS). Regulations are available at http://www.hhs.gov/ohrp/. Laws and regulations governing researchers in other countries must be observed, but experiments should be designed to conform to the intent of the HHS regulations as far as possible.

In relation to the subject matter of the *Journal*, experiments involving taste and food quality evaluation and consumer acceptance are exempt from the above regulations [CFR 46.101 (b) (6)]. However, it should be noted that this would not exempt studies in which extracts, isolates, pure compounds, etc., obtained from conventional food sources are subjected to such evaluation.

JAFC will reject any manuscript for which there is reason to believe that animals have been subjected to unnecessary pain or distress or when informed consent of human subjects is absent or incomplete.

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Any graphic (figure chart, scheme, or equation) that has appeared in an earlier publication should include a credit line citing the original source. Authors are responsible for obtaining written permission to re-use this material.

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Manuscripts, graphics, supporting information, and required forms, as well as manuscript revisions, must all be submitted in digital format through ACS Paragon Plus, which requires an ACS ID to log in. Registering for an ACS ID is fast, free, and does not require an ACS membership. Please refer to Appendix 1 for additional information on preparing your submission

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All manuscripts submitted are reviewed and handled by the Editor-in-Chief or Executive Editors, or assigned to one of the Associate Editors. The Associate Editor and Editorial Assistant are then responsible for the assigned manuscripts, including evaluating the content and format of the paper, selecting reviewers, monitoring the progress of the review process, evaluating the comments of reviewers and forwarding them to the authors for their response,

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Typically, three reviewers are selected per paper on the basis of the subject matter, available expertise, and the Editor's knowledge of the field. Authors must submit the names and addresses (including e-mail addresses) of at least four potential reviewers who do not have conflicts of interest with the authors or manuscript content; however, the Editors are under no obligation to use specific individuals. Reviewers are normally asked to provide their assessments within two to three weeks. Anonymous copies of the reviews and the Editor's decision regarding the acceptability of the manuscript are sent to the corresponding author. If the reviewers' evaluations of the manuscript disagree, or if reviewer's and Editor's comments are not satisfactorily addressed by the authors, the Editor may reject the manuscript or select additional reviewers. These additional reviews are used by the Editor to assist in reaching the final decision regarding disposition of the manuscript.

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Articles may be retracted for scientific or ethical reasons and may be requested by the article author(s) or by the journal Editor(s), but are ultimately published at the discretion of the Editor. Articles that contain seriously flawed or erroneous data such that their findings and conclusions cannot be relied upon may be retracted in order to correct the scientific record. When an article is retracted, a notice of Retraction will be published containing information about the reason for the Retraction. The originally published article will remain online except in extraordinary circumstances (e.g. where deemed legally necessary, or if the availability of the published content poses public health risks).

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Expressions of Concern may be issued at the discretion of the Editor if:

there is inconclusive evidence of research or publication misconduct by the authors;

there is evidence that the findings are unreliable but the authors' institution will not investigate the case;

an investigation into alleged misconduct related to the publication either has not been, or would not be, fair and impartial or conclusive;

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Appendix 2: Preparing Graphics

Resolution

Digital graphics pasted into manuscripts should have the following minimum resolutions:

Black and white line art, 1200 dpi

Grayscale art, 600 dpi

Color art, 300 dpi

Size

Graphics must fit a one- or two-column format. Single-column graphics can be sized up to 240 points wide (3.33 in.) and double-column graphics must be sized between 300 and 504 points (4.167 in. and 7 in.). The maximum depth for all graphics is 660 points (9.167 in.) including the caption (allow 12 pts. For each line of caption text). Lettering should be no smaller than 4.5 points in the final published format. The text should be legible when the graphic is viewed full-size. Helvetica or Arial fonts work well for lettering. Lines should be no thinner than 0.5 point.

Color

Color may be used to enhance the clarity of complex structures, figures, spectra, and schemes, etc., and color reproduction of graphics is provided at no additional cost to the author. Graphics intended to appear in black and white or grayscale should not be submitted in color.

Type of Graphics

Table of Contents (TOC)/Abstract Graphic

Consult the Guidelines for Table of Contents/Abstract Graphics for specifications.

Our team of subject-matter experts and graphical designers can also help generate a compelling TOC graphic to convey your key findings. Learn more about our Graphical Abstract service.

Figures

A caption giving the figure number and a brief description must be included below each figure. The caption should be understandable without reference to the text. It is preferable to place any key to symbols used in the artwork itself, not in the caption. Ensure that any symbols and abbreviations used in the text agree with those in the artwork.

Charts

Charts (groups of structures that do not show reactions) may have a brief caption describing their contents.

Tables

Each table must have a brief (one phrase or sentence) title that describes the contents. The title should be understandable without reference to the text. Details should be put in footnotes, not in the title. Tables should be used when the data cannot be presented clearly in the narrative, when many numbers must be presented, or when more meaningful inter-relationships can be conveyed by the tabular format. Tables should supplement, not duplicate, information presented in the text and figures. Tables should be simple and concise.

Schemes

Each scheme (sequences of reactions) may have a brief caption describing its contents.

Chemical Structures

Chemical structures should be produced with the use of a drawing program such as ChemDraw.

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Image files should be submitted as TIF, JPG, PNG or EPS files with a resolution of at least 300 dpi for pixel-based images. Images should be 8.19 in. wide × 10.00 in. high (or 20.80 cm × 25.40 cm). Please note that the journal title will cover the top 2 in. (5.08 cm) of the image. Authors should submit the cover image, along with a short, clear legend explaining the image, as supplementary files to ACS Paragon Plus with their revised manuscript.

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