

UNIVERSIDADE FEDERAL DO MARANHÃO – UFMA CENTRO DE CIÊNCIAS SOCIAIS, SAÚDE E TECNOLOGIA – CCSST CURSO DE ENGENHARIA DE ALIMENTOS

BIANCA ALMEIDA DE SOUZA

FARINHA DAS PARTES NÃO CONVENCIONAIS DE HIBISCUS: CARACTERIZAÇÃO E ESTABILIDADE DE ARMAZENAMENTO

BIANCA ALMEIDA DE SOUZA

FARINHA DAS PARTES NÃO CONVENCIONAIS DE HIBISCUS: CARACTERIZAÇÃO E ESTABILIDADE DE ARMAZENAMENTO

Trabalho de Conclusão de Curso apresentado ao Curso de Engenharia de Alimentos da Universidade Federal do Maranhão/CCSST, para obtenção de grau de Bacharela em Engenharia de Alimentos.

Orientadora: Prof.^a Dra. Tatiana de Oliveira Lemos

IMPERATRIZ – MA

Ficha gerada por meio do SIGAA/Biblioteca com dados fornecidos pelo(a) autor(a). Diretoria Integrada de Bibliotecas/UFMA

Almeida de Souza, Bianca.

Farinha das partes não convencionais de Hibiscus: caracterização e estabilidade de armazenamento / Bianca Almeida de Souza. - 2022.

70 f.

Orientador(a): Tatiana de Oliveira Lemos. Curso de Engenharia de Alimentos, Universidade Federal do Maranhão, Imperatriz - MA, 2022.

1. Caules. 2. Ferro. 3. Folhas. 4. Proteínas. 5. Vinagreira. I. de Oliveira Lemos, Tatiana. II. Título.

BIANCA ALMEIDA DE SOUZA

FARINHA DAS PARTES NÃO CONVENCIONAIS DE HIBISCUS: CARACTERIZAÇÃO E ESTABILIDADE DE ARMAZENAMENTO

Trabalho de Conclusão de Curso apresentado ao Curso de Engenharia de Alimentos da Universidade Federal do Maranhão/CCSST, para obtenção de grau de Bacharela em Engenharia de Alimentos.

Orientadora: Prof.ª Dra. Tatiana de Oliveira Lemos

BANCA EXAMINADORA

Prof.^a Dra. Tatiana de Oliveira Lemos.

Universidade Federal do Maranhão/CCSST (Orientadora)

Prof.^a Dra. Ana Lúcia Fernandes Pereira

Universidade Federal do Maranhão/CCSST (Membro)

Prof.^a Dra. Anida Maria Moraes Gomes

Faculdade Laboro (Membro)

SUMÁRIO

Introdução	3
Metodologia	7
Hortaliça não-convencional	7
Produção da farinha de vinagreira	7
Caracterização da farinha de vinagreira	7
Umidade, pH, Acidez total titulável (ATT)	7
Atividade de água	7
Valor energético	8
Composição nutricional	8
Vitamina C	8
Contagem de coliformes totais e fecais	8
Pesquisa de Salmonella	8
Contagem de bolores e leveduras	9
Estabilidade da farinha de vinagreira	9
Resultados e Discussão	9
Caracterização da farinha de vinagreira	9
Estudo da estabilidade da farinha de vinagreira	12
Conclusão	16
Referências	19

FARINHA DAS PARTES NÃO CONVENCIONAIS DE HIBISCUS: CARACTERIZAÇÃO E ESTABILIDADE DE ARMAZENAMENTO

Bianca Almeida de Souza¹ e Tatiana de Oliveira Lemos¹.

¹ Universidade Federal do Maranhão, Imperatriz (MA), Brasil.

Resumo

A vinagreira (Hibiscus sabdariffa L.) é uma PANC com relevante potencial nutricional, porém suas folhas e caules são altamente perecíveis. Assim, a desidratação para produção de farinha a partir das folhas e caules desta é uma alternativa promissora. O objetivo da pesquisa foi avaliar as características de identidade e qualidade da farinha de vinagreira e verificar a influência do tipo de embalagem durante o armazenamento. Foram realizadas análises para composição nutricional da farinha: valor energético, carboidratos, proteínas, lipídios, cinzas, ferro, sódio, potássio e vitamina C. Para a estabilidade, a farinha foi acondicionada em garrafa PET (EMB1) e em saco laminado PET metalizado/PEBD sem vácuo (EMB2), armazenadas por 90 dias, com análises a cada 30 dias de: atividade de água (Aa), pH, acidez total titulável (ATT), contagem de coliformes totais e fecais, bolores e leveduras e pesquisa de Salmonella. A composição nutricional determinada foi: 333,73 kcal (valor energético); 60,50 g/100 g (carboidratos); 17,72 g/100 g (proteínas); 2,65 g/100 g (lipídios); 7,67 g/100 g (cinzas); 238,30 mg/100 g (sódio); 143,13 mg/100 g (ferro); 77,99 mg/100 g (vitamina C). Os resultados obtidos durante o armazenamento foram, respectivamente para EMB1 e EMB2: 0,52-0,56 e 0,35-0,48 (Aa); 2,61-2,66 e 2,53-2,69 (pH); 8,13-9,05 e 9,11-9,60 (ATT). Foram obtidas contagens de coliformes totais e fecais < 3 NMP/g e ausência de Salmonella. As contagens de bolores e leveduras atenderam ao limite microbiológico regulatório. Assim, a farinha obteve uma boa

qualidade nutricional e o tipo de embalagem não influenciou em sua segurança. Mas, nas características de identidade e qualidade do produto.

Palavras-chave: Vinagreira, Folhas, Caules, Proteínas, Ferro, Estabilidade.

Introdução

A vinagreira (*Hibiscus sabdariffa L.*) é uma planta alimentícia não convencional (PANC), pertencente à família Malvaceae, com potencial nutricional, contendo proteínas, carboidratos, vitamina C, β-caroteno e ferro [1]. A parte mais usada dessa PANC é o cálice, sendo as folhas e os caules subutilizados. Geralmente, as folhas e os caules são descartados, exceto na África, onde as folhas são utilizadas como ingredientes em sopas e molhos, e no Brasil, onde as folhas são utilizadas na preparação de um alimento típico da gastronomia do estado do Maranhão, chamado arroz de cuxá [2].

As folhas e os caules frescos da vinagreira são perecíveis, o que torna um desafio o armazenamento a longo prazo dessas partes não convencionais de *Hibiscus sabdariffa* para aproveitamento, devido a alta atividade de água dessas, a indisponibilidade de instalações de armazenamento a frio e a possibilidade de deterioração. Consequentemente, a aplicação de uma tecnologia de conservação para aumentar a vida útil desse alimento, como a desidratação, pode permitir seu consumo a longo prazo e facilitar o manuseio, transporte e armazenamento [3].

A partir da tecnologia convencional da desidratação é possível produzir farinha de vegetais como uma alternativa para o aproveitamento das partes não convencionais desses e para a agregação de valor comercial a essas matrizes alimentares [4]. Além disso, a produção de farinhas vegetais vem ganhando destaque na indústria brasileira, devido ao crescente interesse por produtos com melhora do perfil de nutrientes e por promover o aumento de vida útil a esses alimentos naturalmente perecíveis, sendo esta uma alternativa

para o aproveitamento integral dos vegetais [5]. Sendo a farinha, o produto resultante das partes comestíveis de uma ou mais espécies de cereais, leguminosas, frutos, sementes, tubérculos e rizomas por moagem e/ou outros processos tecnológicos que garantam a segurança no processamento de alimentos [6].

Todos os alimentos apresentam um tempo de vida útil que é caracterizado pela sua estabilidade nutricional, sensorial, química, física e microbiológica. Entende-se por estabilidade de um produto o período no qual ele pode ser consumido de forma que essas características estejam conservadas [7]. Para garantir a qualidade e segurança de um produto desidratado é importante armazená-lo em embalagem adequada, já que esta é a principal barreira de proteção entre o alimento e o ambiente externo. A embalagem tem como função evitar o processo de degradação que ocorre devido a interação do alimento com a luz, umidade, variações de temperatura e oxigênio, por exemplo. Além de proteger o alimento contra agentes deteriorantes, é uma barreira contra agentes infectantes e sujidades [8].

O uso de embalagens plásticas em alimentos vem crescendo cerca de 4 a 7% ao ano. Dentre os polímeros mais utilizados em embalagens plásticas temos o politereftalato de etileno – PET, polipropileno – PP, polietileno de baixa densidade – PEBD e o polietileno de baixa densidade – PEAD [9]. Para o armazenamento de farinhas, as embalagens utilizadas no presente estudo, garrafa PET e laminado PET metalizado/PEBD, foram baseadas em estudos prévios e nas embalagens já usadas comercialmente, para produtos similares. Como exemplo temos a farinha de mandioca envasada em garrafa PET abordada no trabalho de Junior e Cruz [10].

O objetivo da pesquisa foi avaliar as características de identidade e qualidade da farinha de vinagreira (*Hibiscus sabdariffa L.*) e verificar a influência do tipo de embalagem utilizada, nas características de identidade e qualidade da farinha durante o

armazenamento. O estudo tem uma patente depositada no INPI no Brasil com número de registro de BR 10 2019 011320 0.

Metodologia

Hortaliça não-convencional

A hortaliça não-convencional vinagreira (*Hibiscus sabdariffa L.*) (Fig. 1) utilizada no presente estudo foi cedida pela Associação dos Agricultores Familiares do Maranhão (AGRIMA) do município de Imperatriz – MA.

Produção da farinha de vinagreira

A farinha de vinagreira foi produzida conforme processo tecnológico descrito na patente de invenção BR 10 2019 011320 0. A formulação utilizada para a composição da farinha de vinagreira foi de 50% de farinha das folhas de vinagreira e 50% de farinha dos caules de vinagreira, visando o uso integral das partes não convencionais da planta.

Após a produção, a farinha (200 g) foi acondicionada nas seguintes embalagens: garrafa PET (Fig. 2) e saco laminado PET metalizado/PEBD sem vácuo (Fig. 3).

Caracterização da farinha de vinagreira

Antes do acondicionamento nas embalagens, a farinha de vinagreira foi submetida às análises a seguir.

pH, Acidez total titulável (ATT)

As análises foram realizadas conforme metodologias descritas pelo Instituto Adolf Lutz [11].

Atividade de água

A atividade de água foi medida por método direto, através de um higrômetro baseado em psicrometria de (Decagon – Aqualab), por ponto de orvalho com controle interno da temperatura da amostra, com resolução de 0,001Aa, sob temperatura de 24 ± 1°C.

Valor energético

Determinou-se com base na composição da farinha de vinagreira utilizando os fatores de conversão de Atwater: 4 kcal. g-1 (carboidratos), 4 kcal.g-1 (proteínas) e 9 kcal.g-1 (lipídios) [12].

Composição nutricional

As análises de carboidratos, proteínas, lipídios, cinzas, ferro, sódio e potássio, conforme metodologias descritas pelo Instituto Adolf Lutz [11].

Vitamina C

O teor de vitamina C foi determinado pelo método 2,6 diclorofenol indofenol segundo [13]. A leitura foi realizada em espectrofotômetro a 520 nm. Os resultados foram expressos em mg.100g⁻¹.

Contagem de coliformes totais e fecais

A contagem de coliformes totais e fecais foi realizada de acordo com o método do NMP técnica de diluição múltipla [14].

Pesquisa de Salmonella

A pesquisa de *Salmonella* foi realizada com o kit analítico 3M Petrifilm Salmonella Express System [15].

Contagem de bolores e leveduras

A contagem de bolores e leveduras foi realizada com o kit analítico 3M Yeast and Mold Count Plate [15].

Estabilidade da farinha de vinagreira

A estabilidade da farinha de vinagreira acondicionada em garrafa PET e em saco laminado PET metalizado/PEBD sem vácuo foi avaliada por um período de 90 dias. A cada tempo de armazenamento (0, 30, 60, 90) foram realizadas as análises de umidade, atividade de água, pH, ATT, cor, contagem de coliformes totais e fecais, pesquisa de Salmonella e contagem de bolores e leveduras. Avaliado de acordo com a análise estatística teste t de Student's (p >0,05)

Resultados e Discussão

Caracterização da farinha de vinagreira

De acordo com a Tabela 1, o valor energético obtido para a farinha de vinagreira, foi de 336,73 kcal/100g, maior que o obtido por Almoraie [17] na farinha de trigo suplementada com farinha de noz, onde foi verificado que a adição melhorou em até 50% o valor nutricional dos pães. Considerando que a declaração de nutrientes obrigatórios no rótulo dos alimentos, deve ser feita também em % de valores diários (%VD), em uma dieta de 2.000 kcal, temos que 100 g da farinha de vinagreira possuem 17% do VD para o valor energético [18].

O conteúdo de carboidratos avaliado na farinha de *Hibiscus* foi de 60,50 g/100 g. Este valor é superior ao observado na farinha das folhas de mandioca que foi de 22,27 g/100 g e na farinha de okara que teve uma média de 32,10 g/100 g de carboidratos [19, 20]. Considerando que a declaração de nutrientes obrigatórios no rótulo dos alimentos,

deve ser feita também em % de valores diários (%VD) e o valor diário de referência de carboidratos para uma dieta adulta adequada, é de 300 g, temos que 100 g da farinha de vinagreira possuem 20 % do VD para carboidratos [18].

O teor de proteínas da farinha de vinagreira foi de 17,72 g/100 g, e merece atenção, já que nos últimos anos houve um crescente interesse sobre a inserção de proteínas vegetais em produtos alimentícios. Nas farinhas vegetais, a fração proteica pode ser explorada como ingrediente nutricional em produtos alimentícios processados, decorrente da atual tendência de alimentos associados à saúde e bem-estar [21]. Em comparação com outros estudos, temos 2,87 g/100 g de proteínas para farinha de jenipapo e 11,76 g/100 g de proteínas para a farinha de casca de maracujá, teores menores que o observado na farinha de vinagreira, [20,19,22]. Com base nos valores obtidos de proteínas para a farinha de vinagreira e o valor diário de referência de proteínas para fins de rotulagem nutricional dos alimentos em geral, que é de 50 g, temos que 100 g de farinha de vinagreira possuem 35% do VD para proteínas [18].

Já o teor de lipídios para a farinha de vinagreira foi de 2,65 g/100 g. Menores teores de lipídios, quando comparado aos teores da farinha de vinagreira, foi verificado na farinha de jenipapo, 0,67 g/100 g, e na farinha de casca de maracujá, 1,64 g/100 g [16, 20]. Já a farinha de okara apresentou um teor de lipídios de 11,9 g/100 g, maior que o teor da farinha de vinagreira [22]. Considerando os valores obtidos para a farinha de vinagreira e o valor diário de referência de gorduras totais para fins de rotulagem nutricional dos alimentos em geral, que é de 65 g, temos que 100 g da farinha de vinagreira possuem 4% do VD para gorduras totais [18].

Para a farinha de vinagreira o teor de cinzas obtido foi de 7,67 g/100 g de cinzas. Teores de cinzas inferiores aos obtidos para a farinha de vinagreira, foram obtidos para a farinha de jenipapo (4,04 g/100 g) e para a farinha de okara (3,0 g/100 g) [16, 22].

Enquanto a farinha de casca de maracujá obteve teor de cinzas superior (8,13 g/100 g) ao da farinha de vinagreira [20]. O teor de cinzas máximo para a farinha de trigo é de 1,4 g/100 g, menor que o observado na farinha de vinagreira [8].

O teor de sódio obtido para a farinha de vinagreira foi de 238,30 mg/100 g. Este valor é superior ao da farinha de trigo que foi de 1,0 mg/100 g, e inferior ao teor de sódio da farinha de *Talinum paniculatum*, que foi de 891,67 mg/100 g [23,21]. Amplamente difundido na natureza, o sódio, no organismo humano, é o eletrólito responsável pela manutenção do volume e osmolaridade do fluido extracelular, contribuindo para a manutenção do equilíbrio ácido-básico, absorção de nutrientes, transmissão nervosa e contração muscular. Contudo, o alto consumo de sódio na dieta é considerado um fator de risco que favorece o desenvolvimento das doenças crônicas não transmissíveis [24]. Conforme os valores obtidos para a farinha de vinagreiras e o valor diário de referência de sódio para fins de rotulagem nutricional dos alimentos em geral, que é de 2.000 mg, temos que 100 g de farinha de vinagreira representam 12% do VD para sódio [18].

O teor de ferro obtido para a farinha de vinagreira foi de 143,13 mg/100 g. O ferro é indispensável para o desenvolvimento correto de diversas funções fisiológicas. Segundo a ANVISA [25], o recomendado é a ingestão de 14,0 mg desse mineral diariamente por adultos. Assim, a farinha de vinagreira apresenta valor consideravelmente, superior a esta recomendação. De acordo com os valores obtidos para a farinha de vinagreira e o valor diário de referência (VDR) de ferro para fins de rotulagem nutricional dos alimentos em geral, que é de 14 mg, temos que para atender VDR se faz necessário o consumo de 9,78 g de farinha de vinagreira [18]. No estudo de Schveitzer et al. [26] foram obtidos teores de ferro de 39,1 mg/100 g em farinha de inflorescência de brócolis, 64,0 mg/100 g em farinha de cenoura e 16,4 mg/100 g em farinha de repolho, teores menores que o obtido na farinha de vinagreira.

O teor de vitamina C obtido para a farinha de vinagreira de 77,99 mg/100 g, apresenta-se como um dado relevante, em comparação com outros tipos de farinhas vegetais, tais como, 18,99 mg/100 g obtidos na farinha de jenipapo e 56,6 mg/100 g obtidos na farinha de folhas de mandioca [16, 19]. A vitamina C possui função antioxidante e é encontrada naturalmente, em frutas e vegetais. Além disso, é muito sensível ao processamento e condições de armazenamento, podendo sofrer significativa degradação durante esses períodos. Assim, a sua retenção é considerada como um índice de conservação de alimentos e da qualidade nutricional do mesmo [27]. Conforme os valores obtidos para a farinha de vinagreiras e o valor diário de referência de vitamina C para fins de rotulagem nutricional dos alimentos em geral, que é de 100 mg, temos que 100 g de farinha de vinagreira representam 78% do VD para vitamina C [18].

Estudo da estabilidade da farinha de vinagreira

A Tabela 2 apresenta os resultados das análises das características de identidade e qualidade da farinha de vinagreira acondicionada em garrafa PET (EMB1) e saco laminado PET metalizado/PEBD sem vácuo (EMB2), durante o armazenamento por 90 dias a temperatura ambiente (25 °C). O parâmetro de atividade de água variou para EMB1 de 0,52 a 0,56 e para EMB2 de 0,35 a 0,48. No entanto, os valores obtidos foram menores que 0,60, o que pode garantir uma maior vida útil à farinha de vinagreira, devido à menor disponibilidade de água livre para o crescimento microbiano [28]. Considerando a atividade de água com o tipo de embalagem, foi observado que houve diferença significativa entre EMB1 e EMB2 e que EMB1 obteve uma menor atividade de água. A farinha foi obtida por metodologia padronizada, tendo como referência o valor de atividade de água abaixo de 0,60 para finalização da secagem. Seth e Sarkar [29] afirmaram que vários fatores podem afetar a difusividade efetiva do produto durante o

processo de secagem. Fatores esses que podem estar relacionados às etapas de obtenção da farinha, tais como, a geometria do material, onde a etapa de trituração influencia a geometria das folhas e caules triturados, podendo interferir na difusividade do material e consequentemente, na remoção da água desse [30]. Sendo assim, embora as folhas e caules da vinagreira desidratados atendam ao critério de finalização da secagem, não necessariamente apresentaram a mesma atividade de água, uma vez que cada processo pode ter um desempenho diferente devido ao fator mencionado. Além disso, cada embalagem possui uma taxa de permeabilidade ao vapor de água diferente, que também podem ter contribuído para a diferença no comportamento da atividade de água entre as embalagens. Hemery et al. [31] verificaram que a atividade de água da farinha de trigo fortificada embalada em saco PET/alumínio não mudou significativamente, durante 6 meses de armazenamento a temperatura de 25 °C com umidade relativa do ar (UR) de 65% e 85% (0.64 ± 0.02). Já as amostras de farinha de trigo fortificada embaladas em sacos de papel, armazenadas durante 6 meses, apresentaram variações de atividade de água com a UR, onde as amostras de farinha de trigo fortificada armazenadas a temperatura de 25 °C e 65% UR sofreram uma redução dos valores de atividade de água. Enquanto as amostras de farinha armazenadas a temperatura de 25 °C e 85% UR apresentaram aumento significativo da atividade de água, comportamento esse similar ao observado no presente estudo [31].

Entre as embalagens, verificou-se que não houve diferença significativa, exceto em T30, para o parâmetro pH, sendo o menor valor encontrado para EMB2 (2,53). Alcântara et al [32] encontraram valores superiores ao descrito para a farinha de vinagreira, ao analisar o pH da farinha do pedúnculo do caju (4,15) e da casca do maracujá (3,77), o que pode ser justificado pela característica de cada matéria-prima. Naturalmente, a vinagreira possui em sua composição ácidos orgânicos, como os ácidos oxálico, málico,

shiquímico e fumárico, com destaque para o ácido málico encontrado em maior concentração (9,10 \pm 0,09 g/100 g), que podem colaborar para o baixo valor de pH da farinha [33].

Quanto à acidez total titulável (ATT), observou-se que, comparando o parâmetro entre as embalagens, não houve diferença significativa, exceto para T90, com a maior média (9,60) para EMB2. Silva et al. [28] obtiveram valor de ATT em farinha de *Talinum paniculatum* inferior aos apresentados neste estudo. Sabe-se que a presença e a natureza dos ácidos nos alimentos influenciam o crescimento microbiano por colaborar na redução do pH [33].

A Tabela 3 apresenta os resultados da avaliação da segurança e qualidade microbiológica da farinha de vinagreira acondicionada em garrafa PET (EMB1) e em saco laminado PET metalizado/PEBD sem vácuo (EMB2), durante o armazenamento por 90 dias a temperatura de 25 °C. As análises foram realizadas durante o período de 90 dias, em intervalos de 30 dias a partir do tempo T0, obtendo valores periódicos para contagem de coliformes totais, contagem de coliformes fecais, pesquisa de Salmonella e contagem de bolores e leveduras.

De acordo com os resultados obtidos, durante o armazenamento das farinhas acondicionadas em EMB1 e EMB2, as contagens de coliformes totais e fecais foram menores que 10 UFC/g e com ausência de *Salmonella*, o que garante a inocuidade do produto. Os resultados obtidos para as farinhas podem ser justificados pelos valores de pH inferiores a 4,5 aliados ao conteúdo de ácidos orgânicos, a Aw menor que 0,60 e a presença de antimicrobianos naturais da vinagreira que impedem o crescimento da maioria das bactérias patogênicas [34]. Sendo assim, temos que as farinhas acondicionadas em EMB1 e EMB2 atenderam ao limite microbiológico estabelecido pela

legislação brasileira para a categoria 2, hortaliças, raízes, tubérculos, fungos comestíveis e derivados, categoria específica d, secos, desidratados ou liofilizados [35].

Com relação à contagem de bolores e leveduras, os resultados obtidos para a farinha envasada em EMB1 e EMB2, foram menores que 10 UFC/g, exceto em T0 (EMB1 e EMB2) e T60 (EMB1). Os bolores e as leveduras são mais tolerantes a pH ácidos e baixa atividade de água do que as bactérias, por essa razão podem deteriorar a farinha. A vida útil da farinha é dependente da multiplicação da microbiota contaminante, quanto maior a carga microbiana inicial, menor a vida útil do produto, devido ao aumento da atividade microbiana. Sendo assim, com relação a carga microbiana necessária para a deterioração de alimentos, há um consenso na literatura de que em contagens microbianas acerca de aproximadamente 10⁷ UFC/g ou mL de produto, os micro-organismos são capazes de produzir metabólitos e/ou alterarem o alimento de forma a tornar perceptível a deterioração [34, 36].

Santos et al. [37] produziram e analisaram farinha de banana verde armazenada por 90 dias e constataram que as contagens de coliformes totais, termotolerantes e bolores e leveduras, atenderam aos limites microbiológicos estabelecidos pela legislação brasileira, resultado este similar ao da presente pesquisa. Já Wanderley [9] em seu estudo realizou avaliação microbiológica da farinha de batata-doce cultivar Beauregard, durante 180 dias de armazenamento, e verificou ausência de Salmonella sp. nas amostras avaliadas, exceto na amostra acondicionada em embalagem de polietileno de alta densidade transparente, resultado este que difere do obtido no presente estudo. Além disso, Wanderley verificou em sua pesquisa que as amostras de farinha de batata doce obtiveram contagem de bolores e leveduras < 10 UFC/g em até 90 dias de armazenamento [9], resultado este que difere do obtido na avaliação microbiológica da farinha de vinagreira.

Os resultados da avaliação microbiológica da farinha de vinagreira armazenada por 90 dias a temperatura de 25 °C demonstraram que as embalagens utilizadas para acondicionar o produto, não comprometeram a segurança e a qualidade microbiológica do produto durante o armazenamento.

Conclusão

Com base nas avaliações para a composição nutricional da farinha de vinagreira podemos afirmar que é uma ótima fonte de proteína, ferro e vitamina C, configurando assim, uma fonte alimentar alternativa para a inclusão de nutrientes na dieta.

A partir dos resultados obtidos para a avaliação da estabilidade da farinha de vinagreira, foi possível concluir que o parâmetro de atividade de água foi influenciado pelo tipo de embalagem utilizada no acondicionamento do produto durante o armazenamento. Já os parâmetros de pH e ATT sofreram influência do tipo de embalagem em T30 e T90, respectivamente. No entanto, o tipo de embalagem não influenciou de forma negativa a estabilidade da farinha.

Considerando os parâmetros analisados para EMB1 e EMB2, temos que os resultados obtidos atenderem as referências da literatura para o controle do desenvolvimento microbiano, destaque para atividade de água inferior a 0,60. No entanto, EMB2 obteve uma menor Aw durante o armazenamento, o que pode garantir uma melhor estabilidade para a farinha de vinagreira. As embalagens utilizadas no acondicionamento do produto não comprometeram a segurança e a qualidade microbiológica da farinha durante o armazenamento por 90 dias a temperatura de 25° C.

Referências

- Llodibia CV, Achebe UA, Arubalueze CU, Ibeh E (2019) A comparative study on morphology, mineral and vitamin compositions of two variants of Hibiscus sabdariffa (L.). Archives of Agriculture and Environmental Science 50-56
- Mendonça GR, Campos RS, Abreu VG, Lemos TO, Pereira ALF (2020) Effect of extract or infusion of leaves of the Hibiscus sabdariffa L. in the production and storage of the beverage blendswith cupuassu: physico-chemical and sensory acceptance. J Food Sci Technol. doi.org/10.1007/s13197-020-04752-4
- Bhatta S, Janezic TS, Ratti C (2020) Freeze-drying of plant-based foods. Foods 9,
 87
- Engel B, Baccar NM, Marquardt I, Oliveira MSR, Rohlfes ALB (2016).
 Tecnologias de atomização e desidratação: alternativas para a produção de farinhas
 a partir de vegetais. Revistas Jovens Pesquisadores, p. 31-44.
 http://dx.doi.org/10.17058/rjp.v6i1.7345
- Couto LA, Coqueiro JS (2020) Desenvolvimento de farinha de quiabo orgânico.
 Profiscientia http://profiscientia.ifmt.edu.br/profiscientia/index.php/profiscientia/article/view/295/171. Acessado em 13 de junho de 2022
- 6. Brasil (2005). Resolução RDC nº 263, de 22 de setembro de 2005. Regulamento técnico para produtos de cereais, amidos, farinhas e farelos. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2005/rdc0263_22_09_2005.html.
 - Acessado em 12 junho 2022
- 7. Reis DS, Neto AF, Ferraz AV, Freitas ST (2017) Produção e estabilidade de conservação de farinha de acerola desidratada em diferentes temperaturas. Braz. J. Food Technol. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1981-67232017000100416&lng=en&nrm=iso. Acessado em 26 março 2021 https://doi.org/10.1590/1981-6723.8315

- 8. SILVA MI (2017) Polpa de um umbu em pó: estabilidade durante armazenamento e aplicação no desenvolvimento de biscoitos. Trabalho de Conclusão de Curso, Instituto Federal de Educação. http://releia.ifsertao-pe.edu.br:8080/jspui/handle/123456789/211. Acessado em 26 março de 2021.
- 9. Wanderley KAA (2017) Caracterização microbiológica e físico-química de farinha de bata-doce cultivar Beauregard armazenada em diferentes embalagens.
 - Dissertação, Universidade Federal do Pernambuco. https://repositorio.ufpe.br/handle/123456789/25578
- 10. Silva Jr AMG, Cruz PJA (2019) Análise das novas tecnologias de produção de farinha de mandioca: Um estudo de caso da agroindústria sabor de Bragança. Trabalho de Conclusão de Curso, Universidade Federal Rural da Amazônia
- Zenebon O, Pascuet NS, Tiglea P (2008) Métodos físico-químicos para análise de alimentos. Instituto Adolfo Lutz, São Paulo, pp 1020
- 12. Brasil (2020). Resolução RDC nº 429, de 8 de outubro de 2020. Rotulagem nutricional dos alimentos embalados http://antigo.anvisa.gov.br/documents/10181/3882585/RDC_429_2020_.pdf/9dc15f3a-db4c-4d3f-90d8-ef4b80537380. Acessado em 12 junho 2022
- 13. Pearson D (1971) The chemical analysis of foods. Fist american, New York, pp 149
- 14. Kornacki JL, Gurtler J B, Stawick B A (2015) Enterobacteriaceae, coliforms, and Escherichia coli as quality and safety indicators. Compendium of methods for the microbiological examination of foods
- 15. Latimer Jr GW (ed) (2016) Official Methods of analysis of AOAC international.
 AOAC International, Maryland

- 16. Cardoso DR, Pinto LIF, Lima MA, Soares IF, Rocha FPS, Viana VGF (2020) Potencial tecnológico e composição de farinha de jenipapo (Genipa americana L) obtida por secagem em convecção. Braz. J. of Develop. 33448-33467. DOI:10.34117/bjdv6n6-050
- 17. Almoraie MN (2019) The effect of walnut flourom the physical and sensory characteristics of wheat bread. International Journal of Food Science. https://doi.org/10.1155/2019/5676205
- 18. Brasil (2020). Instrução Normativa nº 75, de 8 de outubro de 2020. Requisitos técnicos para a declaração de rotulagem nutricional nos alimentos embalados. https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-75-de-8-de-outubro-de-2020-282071143. Acessado em 12 junho 2022
- 19. Trombini FRM, Leonel M (2014) Composição físico-química e propriedades tecnológicas da farinha de folhas de mandioca. Energ. Agric.,76-81
- 20. Costa DS, Santos MS, Neves JF, Egea MB (2016) Como produzir farinha vegetal a partir da secagem do okara. Informe Goiano
- 21. Silva LA, Alves ES, Saqueti BHF, Silva DMB, Raspe DT, Artilha CAF (2019)
 Proteínas Vegetais Como Alimentos Funcionais Revisão. Anais XI EPCC -
 - Encontro Internacional de Produção Científica
- 22. Souza MWS, Ferreira TBO, Vieira LFR (2008) Composição centesimal e propriedades funcionais tecnológicas da farinha da casca do maracujá. Alim. Nutr., 33-36
- 23. Nepa Núcleo de estudos e pesquisas em alimentação (2011) Tabela Brasileira de Composição de Alimentos (TACO) UNICAMP, Campinas
- 24. Cozzolino SMF (2016) Biodisponibilidade de nutrientes. Rev. e atual.

- 25. Brasil (2005). Resolução RDC nº 269, de 22 de setembro de 2005. Regulamento técnico sobre a ingestão diária recomendada (IDR) de proteína, vitaminas e minerais.https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2005/rdc0269_22_09_20 05.html. Acessado em 13 junho 2022
- 26. Schveitzer B, Gabardo GC, Vieira VLS, Oliveira LP, Foppa T (2016)
 Caracterização química das farinhas e hortaliças e de descartes agrícolas. Revista da
 13ª Jornada de pós-graduação e pesquisa
- 27. Home L, Marmitt LG (2019) Métodos para a determinação de vitamina c em diferentes amostras. Revista Destaques Acadêmicos. doi:http://dx.doi.org/10.22410/issn.2176-3070.v11i4a2019.2280
- 28. Silva FC, Neto FESS, Silva MM, Souza BA, Araújo DS, Souza LC, Lemos TO, Pereira ALF, Abreu VKG (2021) Physico-chemical and Technological Functional Properties for Food Applications of Talinun paniculatum Flour for Food Applications. GEINTEC. 10.7198/geintec.v11i1.1467
- 29. Seth D, Sarkar A (2004) A Lumped Parameter Model for Effective Moisture

 Diffusivity in Air Drying of Foods. Food and Bioproducts Processing. p. 183-192.
- 30. Souza TL, Evangelista MEM, Fernandes MMS, Santos KP, Rodrigues JJ (2020)
 Processo de secagem solar e transferência de massa em amostras de mandioca.

Brazilian Journal of Development. p. 86647-86657. 10.34117/bjdv6n11-186

31. Hemery YM, Fontana L, Laillouc A, Jallierd V, Moench-Pfannere R, Avalloneb S, Berger J (2020) Influence of storage conditions and packaging of fortified wheat flour on microbial load and stability of folate and vitamin B12 Food Chemistry. https://doi.org/10.1016/j.fochx.2019.100076

- 32. Alcântara SR, Sousa CAB, Almeida FAC, Gomes JP (2012) Caracterização físico-química das farinhas do pedúnculo do caju e da casca do maracujá. Revista Brasileira de Produtos Agroindustriais.
 http://dx.doi.org/10.15871/1517-8595/rbpa.v15n4p349-355
- 33. Jabeur I, Pereira E, Barros L, Sokovic M, Ferreira, ICFR (2018). Hibiscus sabdariffa L. como uma fonte de nutrientes, compostos bioativos e agentes corantes. V Encontro de Jovens Investigadores do Instituto Politécnico de Bragança. http://hdl.handle.net/10198/14788 Acessado em 27 de agosto de 2021
- 34. Ribeiro BD, Pereira KS, Nascimento RP, Coelho MAZ (2018) Microbiologia industrial. Elsevier
- 35. Brasil (2019) Instrução Normativa N° 60, de 23 de dezembro de 2019. Listas de padrões microbiológicos para alimentos. https://www.in.gov.br/en/web/dou/-/instrucao-normativa-n-60-de-23-de-dezembro-de-2019-235332356. Acessado em 13 junho 2022
- 36. Forsythe SJ (2013) Microbiologia da segurança dos alimentos. Artmed
- 37. Santos JC, Silva GF, Santos JAB, Oliveira Jr AM (2010) Processamento e avaliação da estabilidade da farinha de banana verde. Exacta. https://www.redalyc.org/articulo.oa?id=81016917011 Acessado em 27 de maio de 2021

Tabela 1 Composição nutricional da farinha de vinagreira (Hibiscus sabdariffa)

Parâmetros	Farinha de vinagreira
Valor energético	$336,73 \pm 0,10$
Carboidratos (g/100 g)	$60,50 \pm 0,23$
Proteínas (g/100 g)	$17,72 \pm 0,06$
Lipídios (g/100 g)	$2,65 \pm 0,10$
Cinzas (g/100 g)	$7,67 \pm 0,10$
Sódio (mg/100 g)	$238,30 \pm 7,48$
Ferro (mg/100 g)	$143,13 \pm 8,18$
Vitamina C	$77,99 \pm 0,32$

Todos os valores são representados como Média ±

Desvio padrão n = 3.

Tabela 2 Caracterização da farinha de vinagreira acondicionada em garrafa PET (EMB1) e em saco laminado PET metalizado/PEBD sem vácuo (EMB2), durante o armazenamento por 90 dias a temperatura de 25°C.

Tempo	Aw		pН		ATT (g.100 g ⁻¹)		
	EMB1	EMB2	EMB1	EMB2	EMB1	EMB2	
T0	$0,52 \pm 0,00^{a}$	0.35 ± 0.02^{b}	2,61 ± 0,01°	$2,56 \pm 0,05^{a}$	8,76 ± 0,31 ^a	$9,18 \pm 0,25^{a}$	
T30	$0.54 \pm 0.00^{\circ}$	$0,43 \pm 0,00^{d}$	$2,62 \pm 0,03^{b}$	$2,53 \pm 0,04^{c}$	$8,66 \pm 0,40^{b}$	$9,56 \pm 0,54^{b}$	
T60	$0,53 \pm 0,00^{\rm e}$	$0,\!46 \pm 0,\!01^{\mathrm{f}}$	$2,66 \pm 0,07^{d}$	$2,69 \pm 0,04^d$	$8,13 \pm 0,04^{\circ}$	$9,11 \pm 0,60^{\circ}$	
T90	$0,56 \pm 0,00^{g}$	$0,\!48 \pm 0,\!00^{\rm h}$	$2,60 \pm 0,06^{e}$	$2,69 \pm 0,09^{e}$	$9,05 \pm 0,08^{\rm e}$	$9,60 \pm 0,00^{d}$	

T0: farinha de vinagreira no dia do envase. T30: farinha de vinagreira após 30 dias de envase. T60: farinha de vinagreira após 60 dias de envase. T90: farinha de vinagreira após 90 dias de envase.

Médias seguidas por letras minúsculas diferentes nas colunas diferem entre si pelo teste t de Student's (p < 0.05).

Tabela 3 Avaliação da segurança e qualidade microbiológicas da farinha de vinagreira acondicionada em garrafa PET (EMB1) e em saco laminado PET metalizado/PEBD sem vácuo (EMB2), durante o armazenamento por 90 dias a temperatura de 25 °C

	Coliforn	nes totais	Coliforn	nes fecais	Salmon	ella/25 g	Bolores e	leveduras
Tempo	(NMP/g)		(NMP/g)				(UF	C/ g)
	EMB1	EMB2	EMB1	EMB2	EMB1	EMB2	EMB1	EMB2
Т0	< 3	< 3	< 3	< 3	AUS	AUS	$7,4 \times 10^2$	5,1 x 10 ³
T30	< 3	< 3	< 3	< 3	AUS	AUS	< 10	< 10
T60	< 3	< 3	< 3	< 3	AUS	AUS	$1,2 \times 10^3$	< 10
T90	< 3	< 3	< 3	< 3	AUS	AUS	< 10	<10

EMB1-T0: farinha de vinagreira no dia do envase em garrafa PET. EMB1-T30: farinha de vinagreira após 30 dias de envase em garrafa PET. EMB1-T60: farinha de vinagreira após 60 dias de envase em garrafa PET. EMB1-T90: farinha de vinagreira após 90 dias de envase em garrafa PET. EMB2-T0: farinha de vinagreira no dia do envase em embalagem laminada PET metalizada/PEBD sem vácuo. EMB2-T30: farinha de vinagreira após 30 dias de envase em embalagem laminada PET metalizada/PEBD sem vácuo. EMB2-T60: farinha de vinagreira após 60 dias de envase em embalagem laminada PET metalizada/PEBD sem vácuo. EMB2-T90: farinha de vinagreira após 90 dias de envase em embalagem laminada PET metalizada/PEBD sem vácuo. AUS: Ausência.

Fig 1 Vinagreira (Hibiscus sabdariffa)



Fig 2 Farinha de vinagreira acondicionada em garrafa PET



Fig 3 Farinha de vinagreira acondicionada em saco laminado PET metalizado/PEBD sem vácuo



Normas para os autores do periódico escolhido Revista: Plant Foods for Human Nutrition

Submission guidelines

• Instructions for Authors o

Types of papers

Manuscripts, review papers, short communication.

16 double-spaced page requirement for original ms; 30 pages for review article; 4 pages for short communication. Title page, references, tables and figures should be included in the manuscript body.

Line spacing should be doubled.

The font size regardless of style should be 12pt.

At least five potential reviewers should be provided

o Additional information

Abbreviations should be collected and their explanations should be collected in a list, arranged alphabetically.

o Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

o <u>Title Page</u>

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

The name(s) of the author(s)

The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country

A clear indication and an active e-mail address of the corresponding author

If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration for prospectively registered trials

Trial registration number and date of registration, followed by "retrospectively registered", for retrospectively registered trials

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Statements and Declarations

The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

Competing Interests: Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to "Competing Interests and Funding" below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

o Scientific style

- Please always use internationally accepted signs and symbols for units (SI units).
- Genus and species names should be in italics.

Please use the standard mathematical notation for formulae, symbols etc.:Italic for single letters that denote mathematical constants, variables, and unknown quantities Roman/upright for numerals, operators, and punctuation, and commonly defined functions or abbreviations, e.g., cos, det, e or exp, lim, log, max, min, sin, tan, d (for derivative) Bold for vectors, tensors, and matrices.

o References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

- 1. Negotiation research spans many disciplines [3].
- 2. This result was later contradicted by Becker and Seligman [5].
- 3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The entries in the list should be numbered consecutively. If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

• Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

• Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

• Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

• Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibliography style file sn-basic.bst which is included in the <u>Springer Nature Article Template</u>.

o Tables

All tables are to be numbered using Arabic numerals.

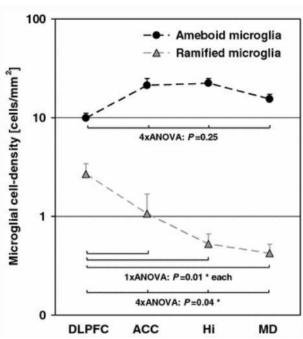
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

o Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

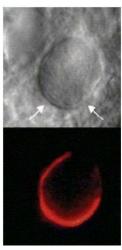
Line Art



• Definition: Black and white graphic with no shading.

- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
 - All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art

Group I * mGlu1a 1199

b mGlu1β 906

mGlu1d 908

mGlu55 301

mGlu5a 1171

mGlu5b 1203

Group III b mGlu3 936

mGlu3A4 535

Group III h mGlu6a 871

mGlu7a 915

mGlu7a 915

mGlu7a 922

mGlu7c 924

mGlu7c 924

mGlu7c 924

mGlu7c 906

mGlu8c 501

• Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.

- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel). Figure Lettering
- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.
 Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
 - No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
 - Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

•

• For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

• If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

- In order to give people of all abilities and disabilities access to the content of your figures, please make sure that
- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1
 - o Supplementary Information (SI)

- Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.
- Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB. Audio, Video, and Animations
- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

• Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

• Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as "Online Resource", e.g., "... as shown in the animation (Online Resource 3)", "... additional data are given in Online Resource 4".
- Name the files consecutively, e.g. "ESM 3.mpg", "ESM 4.pdf".

Captions

• For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting. Accessibility
- In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that
- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

o Ethical Responsibilities of Authors

- This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.
- Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:
- The manuscript should not be submitted to more than one journal for simultaneous consideration.

- The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the reuse of material to avoid the concerns about text-recycling ('self-plagiarism').
- A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').
- Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.
- Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.
- No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.
 - Important note: the journal may use software to screen for plagiarism.
- Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).
- Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.
- Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.

• Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or

- toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).
- Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.
- *All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.
- Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.
- If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:
- If the manuscript is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction:
- - an erratum/correction may be placed with the article
- an expression of concern may be placed with the article - or in severe cases retraction of the article may occur.
- The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is maintained on the platform, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.
- The author's institution may be informed
 A notice of suspected transgression of ethical standards in the peer review system
 may be included as part of the author's and article's bibliographic record.

Fundamental errors

• Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

• Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

o Authorship principles

- These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to. Authorship clarified
- The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted.
- The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

- 1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;
- 2) drafted the work or revised it critically for important intellectual content;
- 3) approved the version to be published; and
- 4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- * Based on/adapted from:
- ICMJE, Defining the Role of Authors and Contributors, Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

- All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).
- The decision whether such information should be included is not only dependent
 on the scope of the journal, but also the scope of the article. Work submitted for
 publication may have implications for public health or general welfare and in
 those cases it is the responsibility of all authors to include the appropriate
 disclosures and declarations. Data transparency
- All authors are requested to make sure that all data and materials as well as
 software application or custom code support their published claims and comply
 with field standards. Please note that journals may have individual policies on
 (sharing) research data in concordance with disciplinary norms and expectations.

Role of the Corresponding Author

- One author is assigned as Corresponding Author and acts on behalf of all coauthors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.
 - The Corresponding Author is responsible for the following requirements:
- ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;

- managing all communication between the Journal and all co-authors, before and after publication;*
- providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;
- making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).
- * The requirement of managing all communication between the journal and all
 co-authors during submission and proofing may be delegated to a Contact or
 Submitting Author. In this case please make sure the Corresponding Author is
 clearly indicated in the manuscript.

Author contributions

- In absence of specific instructions and in research fields where it is possible to
 describe discrete efforts, the Publisher recommends authors to include
 contribution statements in the work that specifies the contribution of every author
 in order to promote transparency. These contributions should be listed at the
 separate title page.
- Examples of such statement(s) are shown below:
- Free text:
- All authors contributed to the study conception and design. Material preparation,
 data collection and analysis were performed by [full name], [full name] and [full
 name]. The first draft of the manuscript was written by [full name] and all authors
 commented on previous versions of the manuscript. All authors read and approved
 the final manuscript.
- Example: CRediT taxonomy:
- Conceptualization: [full name], ...; Methodology: [full name], ...; Formal analysis and investigation: [full name], ...; Writing original draft preparation: [full name, ...]; Writing review and editing: [full name], ...; Funding acquisition: [full name], ...; Resources: [full name], ...; Supervision: [full name],

 For review articles where discrete statements are less applicable a statement should be included who had the idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.
- For articles that are based primarily on the student's dissertation or thesis, it is recommended that the student is usually listed as principal author:

• A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006 Affiliation

• The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship

- Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in Corresponding Author, and/or changes in the sequence of authors are not accepted after acceptance of a manuscript.
- Please note that author names will be published exactly as they appear on the accepted submission!
- Please make sure that the names of all authors are present and correctly spelled,
 and that addresses and affiliations are current.
- Adding and/or deleting authors at revision stage are generally not permitted, but
 in some cases it may be warranted. Reasons for these changes in authorship should
 be explained. Approval of the change during revision is at the discretion of the
 Editor-in-Chief. Please note that journals may have individual policies on adding
 and/or deleting authors during revision stage. Author identification
- Authors are recommended to use their ORCID ID when submitting an article for consideration or acquire an ORCID ID via the submission process.

Deceased or incapacitated authors

• For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to

include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes

• In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality

Authors should treat all communication with the Journal as confidential which
includes correspondence with direct representatives from the Journal such as
Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit
consent has been received to share information.

o Compliance with Ethical Standards

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the abovementioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

o Competing Interests

- Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.
- Editorial Board Members and Editors are required to declare any competing interests and may be excluded from the peer review process if a competing interest exists. In addition, they should exclude themselves from handling manuscripts in cases where there is a competing interest. This may include but is not limited to having previously published with one or more of the authors, and sharing the same institution as one or more of the authors. Where an Editor or Editorial Board Member is on the author list they must declare this in the competing interests section on the submitted manuscript. If they are an author or have any other competing interest regarding a specific manuscript, another Editor or member of the Editorial Board will be assigned to assume responsibility for overseeing peer review. These submissions are subject to the exact same review process as any other manuscript. Editorial Board Members are welcome to submit papers to the journal. These submissions are not given any priority over other manuscripts, and Editorial Board Member status has no bearing on editorial consideration.

- Interests that should be considered and disclosed but are not limited to the following:
- Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.
- Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).
- Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.
- It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."
- Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.
- Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a

subject. Review articles therefore require a disclosure statement. Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

• Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is not added to the manuscript itself. Under 'summary of requirements' (see below) funding information should be included in the 'Declarations' section.

Summary of requirements

- The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.
- Please see the various examples of wording below and revise/customize the sample statements according to your own needs.
- When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.
- Examples of statements to be used when funding has been received:
- Partial financial support was received from [...]
- The research leading to these results received funding from [...] under Grant Agreement No[...].
- This study was funded by [...]
- This work was supported by [...] (Grant numbers [...] and [...]
- Examples of statements to be used when there is no funding:
- The authors did not receive support from any organization for the submitted work.

- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this study.
- No funds, grants, or other support was received.
- Examples of statements to be used when there are interests to declare:
- Financial interests: Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.
- Non-financial interests: Author C is an unpaid member of committee Z.
- Financial interests: The authors declare they have no financial interests.
- Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.
- Financial interests: Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.
- Non-financial interests: none.
- Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.
- Non-financial interests: Author D has served on advisory boards for Company M,
 Company N and Company O.
- Examples of statements to be used when authors have nothing to declare:
- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no competing interests to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any
 organization or entity with any financial interest or non-financial interest in the
 subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed

in this article.

• Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

o Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on

this subject. Authors should check with their institution to make sure they are

complying with the specific requirements of their institution and seek ethics approval

where needed. Authors should be aware to secure informed consent from the

individual (or parent or guardian if the participant is a minor or incapable) See also

section on Informed Consent.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were

used by describing the source of the cell line, including when and from where it was

obtained, whether the cell line has recently been authenticated and by what method.

If cells were bought from a life science company the following need to be given in

the manuscript: name of company (that provided the cells), cell type, number of cell

line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and

contamination of human cell lines. This step will alert authors to possible problems

with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication

Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent

ethics committee (including the name of the ethics committee) approved the study

and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively

similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs

when reporting key biological resources (antibodies, cell lines, model organisms and tools)

in their manuscripts.

Examples:

Organism: Filip1^{tm1a(KOMP)Wtsi} RRID:MMRRC 055641-UCD

Cell Line: RST307 cell line RRID:CVCL C321

Antibody: Luciferase antibody DSHB Cat# LUC-3, RRID:AB 2722109

Plasmid: mRuby3 plasmid RRID: Addgene 104005

Software: ImageJ Version 1.2.4 RRID:SCR 003070

RRIDs are provided by the Resource Identification Portal. Many commonly used

research resources already have designated RRIDs. The portal also provides authors

links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research

study that prospectively assigns human participants or groups of humans to one or

more health-related interventions to evaluate the effects on health outcomes". The

WHO defines health interventions as "A health intervention is an act performed for,

with or on behalf of a person or population whose purpose is to assess, improve,

maintain, promote or modify health, functioning or health conditions" and a health-

related outcome is generally defined as a change in the health of a person or

population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register

prospective clinical trials (phase II to IV trials) in suitable publicly available

repositories. For example www.clinicaltrials.gov or any of the primary registries that

participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the

last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged

to register retrospectively to ensure the complete publication of all results. The trial

registration number (TRN), date of registration and the words 'retrospectively

registered' should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the

<u>EQUATOR Network</u> when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's

Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT) Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

- Examples of statements to be used when ethics approval has been obtained:
 - All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No.).
 - This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No.).
 - Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.
 - The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number:).
- Examples of statements to be used for a retrospective study:
 - Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.
 - This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.
 - This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or

comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

- This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.
- The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
- Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

o Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed

consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

- Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.
- Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent.

Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of

uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for "Consent to participate":

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for "Consent to publish":

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal. Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

o English Language Editing

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

• Getting a fast, free online grammar check.

 Asking a colleague who is proficient in English to review your manuscript for clarity.

• Visiting the English language tutorial which covers the common mistakes when writing in English.

• Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. Springer authors are entitled to a 10% discount on their first submission to either of these services, simply follow the links below.

Free online grammar check English language tutorial

Nature Research Editing Service

American Journal Experts

Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted.

If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

.

为便于编辑和评审专家准确评估您稿件中陈述的研究工作,您需要确保您的英语语言质量足以令人理解。如果您需要英文写作方面的帮助,您可以考虑:

- 获取快速、免费的在线 语法检查。
- 请一位以英语为母语的同事审核您的稿件是否表意清晰。
- 查看一些有关英语写作中常见语言错误的教程。

● 使用专业语言编辑服务,编辑人员会对英语进行润色,以确保您的意思表达清晰,并识别需要您复核的问题。我们的附属机构 Nature Research Editing Service 和合作伙伴 American Journal Experts 即可提供此类服务。

免费在线 语法检查

教程

Nature Research Editing Service

American Journal Experts 请注意,使用语言编辑服务并非在期刊上发表文章的必要条件,同时也并不意味或保证文章将被选中进行同行评议或被接受。

如果您的稿件被接受,在发表之前,我们的文字编辑会检查您的文稿拼写是否规范以及文体是否正式。

_

エディターと査読者があなたの論文を正しく評価するには、使用されている英語の質が十分に高いことが必要とされます。英語での論文執筆に際してサポートが必要な場合には、次のオプションがあります:

- ・高速なオンライン <u>文法チェック</u> を無料で受ける。
- ・英語を母国語とする同僚に、原稿で使用されている英語が明確であるかをチェックしてもらう。
- ・英語で執筆する際のよくある間違いに関する英語のチュートリアルを参照する。
- ・プロの英文校正サービスを利用する。校正者が原稿の意味を明確にしたり、

問題点を指摘し、英語の質を向上させます。 Nature Research Editing Service と American Journal Experts の 2 つは弊社と提携しているサービスです。Springer の 著者は、いずれのサービスも初めて利用する際には 10%の割引を受けることが できます。以下のリンクを参照ください。

無料のオンライン 文法チェック

英語のチュートリアル

Nature Research Editing Service

American Journal Experts 英文校正サービスの利用は、投稿先のジャーナルに掲載されるための条件ではないこと、また論文審査や受理を保証するものではないことに留意してください。

原稿が受理されると、出版前に弊社のコピーエディターがスペルと体裁のチェックを行います。

.

영어 원고의 경우,에디터 및 리뷰어들이 귀하의 원고에 실린 결과물을 정확하 게 평가할 수 있도록,그들이 충분히 이해할 수 있을 만한 수준으로 작성되어야 합니다.만약 영작문과 관련하여 도움을 받기를 원하신다면 다음의 사항들을 고 려하여 주십시오:

- 귀하의 원고의 표현을 명확히 해줄 영어 원어민 동료를 찾아서 리뷰를 의뢰합니다.
- 영어 튜토리얼 페이지에 방문하여 영어로 글을 쓸 때 자주하는 실수들을 확인 합니다.

• 리뷰에 대비하여,원고의 의미를 명확하게 해주고 리뷰에서 요구하는 문제점 들을 식별해서 영문 수준을 향상시켜주는 전문 영문 교정 서비스를 이용합니다.

Nature Research Editing Service $\mathfrak P$ American Journal Experts $\mathfrak P$ \mathfrak

영어 튜토리얼 페이지

Nature Research Editing Service

American Journal Experts

영 문 교 정 서 비 스 는 게 재 를 위 한 요 구 사 항 은 아 니 며 , 해 당 서 비 스 의 이 용 이 피 어 리 뷰 에 논 문 이 선 택 되 거 나 게 재 가 수 락 되 는 것 을 의 미 하 거 나 보 장 하 지 않 습 니 다 .

원고가 수락될 경우,출판 전 저희측 편집자에 의해 원고의 철자 및 문체를 검수 하는 과정을 거치게 됩니다.

o After acceptance

Upon acceptance, your article will be exported to Production to undergo typesetting. Once typesetting is complete, you will receive a link asking you to confirm your affiliation, choose the publishing model for your article as well as arrange rights and payment of any associated publication cost.

Once you have completed this, your article will be processed and you will receive the proofs.

Article publishing agreement

Depending on the ownership of the journal and its policies, you will either grant the Publisher an exclusive licence to publish the article or will be asked to transfer copyright of the article to the Publisher.

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Online publication of color illustrations is free of charge. For color in the print version, authors will be expected to make a contribution towards the extra costs.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

o Open Choice

Open Choice allows you to publish open access in more than 1850 Springer Nature journals, making your research more visible and accessible immediately on publication.

Article processing charges (APCs) vary by journal – view the full list

Benefits:

• Increased researcher engagement: Open Choice enables access by anyone with an internet connection, immediately on publication.

Higher visibility and impact: In Springer hybrid journals, OA articles are accessed
 4 times more often on average, and cited 1.7 more times on average*.

Easy compliance with funder and institutional mandates: Many funders require
open access publishing, and some take compliance into account when assessing
future grant applications.

It is easy to find funding to support open access – please see our funding and support pages for more information.

*) Within the first three years of publication. Springer Nature hybrid journal OA impact analysis, 2018.

Open Choice

Funding and Support pages

Copyright and license term – CC BY

Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License.

Find more about the license agreement

o Research Data Policy and Data Availability Statements

This journal operates a <u>type 3 research data policy</u>. A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Data availability

All original research must include a data availability statement. Data availability statements should include information on where data supporting the results reported in the article can be found, if applicable. Statements should include, where applicable,

hyperlinks to publicly archived datasets analysed or generated during the study. For the purposes of the data availability statement, "data" is defined as the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. When it is not possible to share research data publicly, for instance when individual privacy could be compromised, data availability should still be stated in the manuscript along with any conditions for access. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- 1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
- 2. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
- 3. All data generated or analysed during this study are included in this published article [and its supplementary information files].
- 4. The datasets generated during and/or analysed during the current study are not publicly available due [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.].
- 5. Data sharing not applicable to this article as no datasets were generated or analysed during the current study.
- 6. The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

More templates for data availability statements, including examples of openly available and restricted access datasets, are available here:

Data availability statements

Data repositories

This journal strongly encourages that all datasets on which the conclusions of the paper rely are available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible.

Please see Springer Nature's information on recommended repositories. List of Repositories

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Data citation

The journal also requires that authors cite any publicly available data on which the conclusions of the paper rely. Data citations should include a persistent identifier (such as a DOI), should be included in the reference list using the minimum information recommended by DataCite, and follow journal style. Dataset identifiers including DOIs should be expressed as full URLs.

Research data and peer review

Peer reviewers are encouraged to check the manuscript's Data availability statement, where applicable. They should consider if the authors have complied with the journal's policy on the availability of research data, and whether reasonable effort has been made to make the data that support the findings of the study available for replication or reuse by other researchers. Peer reviewers are entitled to request access to underlying data (and code) when needed for them to perform their evaluation of a manuscript.

If the journal that you're submitting to uses double-blind peer review and you are providing reviewers with access to your data (for example via a repository link, supplementary information or data on request), it is strongly suggested that the authorship in the data is also blinded. There are <u>data repositories that can assist with this</u> and/or will create a link to mask the authorship of your data.

Authors who need help understanding our data sharing policies, help finding a suitable data repository, or help organising and sharing research data can access our <u>Author Support portal</u> for additional guidance.

For more information:

http://www.springernature.com/gp/group/data-policy/faq

• Open access publishing

To find out more about publishing your work Open Access in Plant Foods for Human Nutrition, including information on fees, funding and licenses, visit our <u>Open access publishing page</u>.