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DRYING *TENEBRIO MOLITOR* LARVAE BY VACUUM AND MULTI-FLASH DRYINGS: EFFECTS ON THE MICROBIOTA AND COLOR

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O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi julgado adequado para obtenção do título de mestre em Mestre em Engenharia de Alimentos.

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This work is dedicated to my family.

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“Whether you fly, run, walk or crawl, just make sure you are moving forward."

Martin Luther King

RESUMO

As larvas de *Tenebrio molitor* (YM) tem sido apontadas como uma alternativa às fontes convencionais de proteína animal devido ao seu ótimo valor nutricional e baixo impacto ambiental para a produção. No entanto, alguns estudos relataram alta atividade de água (a_w) e diferentes níveis de contaminação microbiana. Nesse contexto, o branqueamento e a secagem são geralmente empregados para reduzir respectivamente as cargas microbianas e a atividade enzimática e diminuir o teor de umidade e a_w, a fim de prorrogar a vida útil das YM. O objetivo deste estudo foi secar as YM (não branqueadas e branqueadas) até uma a_w entre 0,2 e 0,3 usando a secagem a vácuo contínuo (CVD), a multi-flash drying condutiva I (KMFD) e a multi-flash drying condutiva II (DVD). Efeitos sobre a microbiota e coloração final das YM também foram investigados, bem como curvas cinéticas de secagem. Um objetivo subsequente foi submeter as YM a uma ração contaminada com *Escherichia coli* (teste desafio) e investigar a contaminação e descontaminação usando uma das técnicas de secagem. Foi possível reduzir a a_w de todas as amostras para a faixa alvo. Os tempos de secagem de KMFD e DVD foram mais curtos que os para CVD e, para cada técnica, os tempos usados para as amostras branqueadas para atingir a a_w alvo foram menores do que os das amostras enxaguadas. Para as amostras branqueadas, todas as técnicas mostraram uma redução microbiana satisfatória. Para as amostras não branqueadas, o mesmo nível de redução foi observado para DVD e KMFD, exceto para bactérias ácido lácticas (LAB) e bolores e leveduras (2.5 e 2.3 log ufc/g, respectivamente), e para CVD, exceto para a contagem de células viáveis totais (TVC), LAB e bolores e leveduras (2.0, 4.6 e 2.7 log ufc/g, respectivamente). Através do teste de desafio, usando contaminação artificial, foi possível verificar a eficácia da combinação de branqueamento e CVD na redução da carga de *E. coli* em YM. A secagem CVD preservou melhor a luminosidade das YM. Conclui-se que CVD, DVD e KMFD podem ser usadas para a produção de YM secas. Ainda, branquear as YM antes de secar, proporciona melhores resultados nas características de luminosidade, redução microbiana e tempos de secagem.

Palavras-chave: Insetos comestíveis. Entomofagia. Larvas. Secagem a vácuo. Segurança de alimentos. Teste desafio microbiológico.

RESUMO EXPANDIDO

Introdução

A demanda mundial por alimentos tem crescido significativamente, impulsionada pelo aumento da população e pelo crescimento econômico. Com o esgotamento dos recursos naturais (terra, água e energia), é necessário repensar nossos padrões e hábitos alimentares, principalmente aqueles relacionados ao consumo de carne (VAN HUIS, VAN GURP, DICKE, TAKKEN-KAMINKER e BLUMENFELD-SCHAAP, c 2014). Para cada quilograma de proteína animal de alta qualidade produzida, o gado é alimentado com aproximadamente 6 kg de proteína vegetal. Uma alta quantidade de proteína animal produzida resulta em aumento das emissões de gases de efeito estufa, desmatamento e degradação ambiental (PIMENTEL & PIMENTEL, 2003; VAN HUIS et al., 2014).

Fontes alternativas de proteína que podem diminuir o impacto da criação de animais são plantas com alto teor de proteínas, carne cultivada a partir de células-tronco, algas, fungos e insetos (BHAT & FAYAZ, 2011; FLEURENCE, 1999; VAN HUIS et al., 2014) O hábito de comer insetos como alimento (entomofagia) vem sendo considerado uma das soluções mais adequadas para garantir a segurança alimentar global (ALEXANDRATOS & BRUINSMA, 2012; BELLUCO et al., 2013; McGREW, 2014; RAMOS-ELORDUY, GONZÁLEZ, HERNÁNDEZ , & PINO, 2002; VANTOMME, 2015).

Atualmente, mais de 2000 espécies de insetos são considerados comestíveis e são consumidas por dois bilhões de pessoas em todo o mundo, e alimentos à base de insetos tornaram-se disponíveis recentemente nos Estados Unidos e em alguns países europeus (BELLUCO et al., 2013; JONGEMA, 2015; RAMOS-ELORDUY, 2008; SHELOMI, 2015). Entre os insetos comestíveis, o *Tenebrio molitor* (YM), é um dos insetos mais produzidos para alimentação humana e animal, devido à sua sustentabilidade ambiental e qualidade nutricional atraentes (BARSICS et al., 2017; CAPARROS MEGIDO et al., 2016; CORTES ORTIZ et al., 2016; RUMPOLD & SCHLÜTER, 2013b; VELDKAMP, VAN HUIS, & LAKEMOND, 2012).

No entanto, larvas de YM podem apresentar diferentes níveis de contaminação microbiana. Além disso, larvas frescas de YM apresentam um teor de umidade de até 68% e alta atividade de água (a_w 0,98) que as expõe altamente à deterioração microbiológica, degradação enzimática e não enzimática (reação de Maillard) (LEDL; SCHLEICHER, 1990; RAHMAN , 2007; TAOUKIS; RICHARDSON, 2007). Por cima disso, o YM geralmente pode apresentar até 38% de gorduras, o que as expõe à oxidação lipídica (GHOSH et al., 2017; LENAERTS et al., 2018; ZHENG et al., 2013). O processamento e armazenamento de YM para alimentos ou rações geralmente requerem uma descontaminação prévia para prolongar sua vida útil. Alguns estudos demonstraram a importância de uma etapa preliminar de aquecimento, como o branqueamento, que também é usado para reduzir o número de microrganismos nos insetos antes da secagem (KLUNDER et al., 2012; RUMPOLD et al., 2014; STOOPS et al., 2016; VANDEWEYER et al., 2017a).

A secagem é amplamente utilizada como uma técnica de preservação, uma vez que reduz o teor de umidade e uma grande quantidade de alimentos, inibindo o crescimento de microrganismos, reduzindo a atividade enzimática e reações químicas (GEANKOPLIS, 1998; LABUZA, 1980). Consequentemente, prolonga a vida útil dos produtos alimentícios, além de reduzir os custos de armazenamento e transporte (ALIBAS, 2007; RATTI, 2001). Kröncke et al. (2019) sugeriram que poderia ser mais vantajoso encontrar métodos de secagem mais sustentáveis e potencialmente mais baratos para secagem de YM. Para alimentos que podem sofrer danos ou até perdas de vitaminas quando expostos a altas temperaturas, a secagem a vácuo é uma das técnicas amplamente utilizadas. Aumenta a taxa de evaporação devido à diminuição da temperatura de saturação da água. A manutenção de baixas temperaturas é essencial para produtos termossensíveis, além de estabelecer um ambiente de secagem com baixas concentrações de oxigênio, contribuindo para reduzir as perdas sensoriais e nutricionais dos produtos desidratados (ALIBAS, 2007; REIS, 2014).

Nesse contexto, vale a pena investigar as secagens condutivas a vácuo e multi-flash nas secagens da YM. A secagem com flash múltiplo é um processo de secagem que tem sido usado na desidratação de frutas e vegetais. Nessa técnica, o produto é aquecido à pressão atmosférica até a temperatura desejada e, em seguida, é aplicado um pulso de vácuo, levando à evaporação rápida e, consequentemente, ao resfriamento da amostra (LAURINDO, PORCIUNCULA, ZOTARELLI, 2011). Esta tecnologia de secagem permite produzir alimentos secos com baixo teor de umidade e baixo a_w em tempos de processamento reduzidos (LAURINDO, PORCIUNCULA, ZOTARELLI, 2011; ZOTARELLI; PORCIUNCULA; LAURINDO, 2012).

Objetivos:

Objetivo principal:

Este trabalho tem como objetivo avaliar a influência da secagem condutiva a vácuo (DCV), secagem condutora por múltiplos flashes (KMFD) e secagem condutora por múltiplos flashes (DVD) na cor e microbiota das larvas de Tenebrio molitor.

Objetivos específicos:

a) Determine as curvas de secagem das larvas de T. molitor enxaguadas e branqueadas, secas por DVD e KMFD.

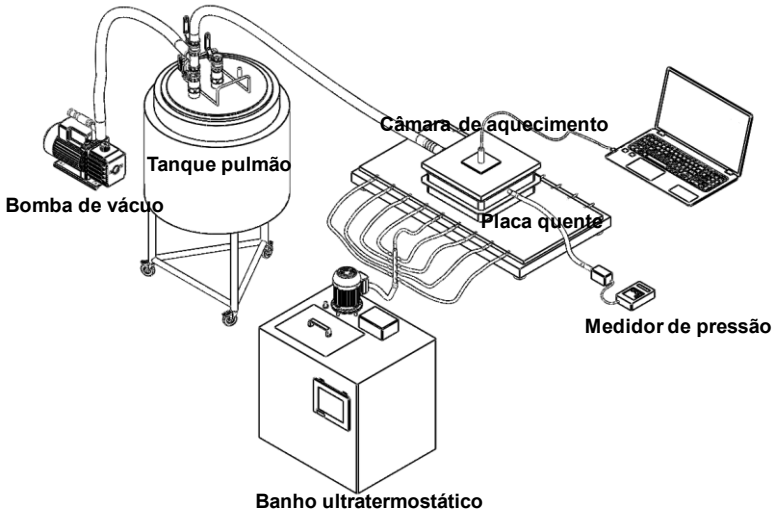
b) Avalie os efeitos de CVD, DVD e KMFD na microbiota e na cor do T. molitor enxaguado e branqueado com aw = 0,3 - 0,2.

c) Submeta a YM a um teste de desafio (contaminação artificial) em um alimento contaminado (farelo de trigo) com Escherichia coli, depois ao tratamento de secagem (DCV) e avalie sua eficácia na descontaminação do patógeno.

Metodologia

Os experimentos foram realizados no Laboratório de Propriedades Físicas dos Alimentos (PROFI) e no Laboratório de Bioprocessos do Departamento de Ciência e Tecnologia de Alimentos da Universidade Federal de Santa Catarina (UFSC). Após obtenção das larvas de *Tenébrio molitor*, uma parte foi separada para a criação e a outra para os experimentos de secagem. Para a parte experimental, as larvas foram primeiramente colocadas em uma geladeira (5 °C por 24h) para dessensibilizá-las. Em seguida, 15 g das larvas foram lavadas (1 L de água destilada, 25 °C, 15s) ou branqueadas (1 L de água destilada fervida, 15s). Finalmente, elas foram colocadas por 1 min em um papel de filtro para remover a água residual. As YM branqueado ou enxaguado (não branqueado) foram colocadas em um recipiente cilíndrico de alumínio (63 mm x 53 mm), que foi colocado na câmara de secagem, recebendo aquecimento condutivo na base, como mostra a figura abaixo. As YM foram secas por três técnicas diferentes: a secagem por vácuo condutivo (CVD), a secagem múltipla condutora I (KMFD) e a secagem múltipla condutora II (DVD). Todos os experimentos de secagem foram realizados em triplicata.

Dispositivo experimental de secagem.



Secagem por vácuo condutivo (CVD)

Para CVD, as amostras foram colocadas na câmara de aquecimento e a pressão foi imediatamente reduzida para 4 kPa (descompressão repentina) e mantida durante todo o processo.

Secagem múltipla condutiva I (KMFD)

Para o KMFD, as amostras foram colocadas na câmara de aquecimento e aquecidas até 60 ° C à pressão atmosférica, depois a pressão foi imediatamente reduzida para 4 kPa (descompressão repentina) e

mantida por 2 minutos. Após os 2 minutos, a pressão atmosférica foi restabelecida e um novo ciclo foi iniciado. Após o quinto ciclo, a pressão foi reduzida para 4 kPa e mantida até o final da secagem.

Secagem múltipla condutiva II (DVD)

Para o DVD, as amostras foram colocadas na câmara de aquecimento e a pressão foi imediatamente reduzida para 4 kPa (descompressão repentina). A intervalos regulares de 10 min, a pressão foi restabelecida à pressão atmosférica e depois imediatamente reduzida novamente, repetidamente até o final da secagem. Nos mesmos intervalos regulares, o recipiente cilíndrico de alumínio e as amostras foram pesados em uma balança analítica e os dados foram usados para traçar as curvas de secagem.

As curvas de secagem foram obtidas pesando-se o recipiente cilíndrico de alumínio e as amostras antes de colocá-lo na câmara de vácuo e após cada pulso de vácuo (KMFD) ou após cada 10 minutos (DVD) ou apenas no final da secagem (CVD).

Para cada experimento de secagem, a umidade (X_{db}), a_w e cor das amostras (frescas, branqueadas e secas) foram medidas em triplicado. O teor de umidade foi determinado pelo método gravimétrico, colocando as amostras em estufa a 105 ° C até peso constante (aproximadamente 24h) (AOAC, 2002).

A a_w foi determinada esmagando as amostras e colocando-as em um higrômetro digital a 25 °C. Os parâmetros de cor das amostras foram determinados por um sistema de visão computacional (CVS), conforme metodologia descrita por Cárdenas-Pérez et al. (2017) com pequenas adaptações. As imagens foram capturadas usando uma câmera (Nikon D5500, Nikon Corporation, Japão) com uma resolução de 4496 × 3000 pixels e uma câmera de fundo branco equipada com luz branca (D65 Lighting Standard). As imagens digitais foram tratadas pelo software ImageJ v. 1.6.0 (National Institutes of Health, EUA). O plug-in do conversor de espaço de cores foi usado para converter cores do sistema RGB para a escala CIELab, os valores L* (brilho) variam de branco (100) para preto (0), a* é definido como uma transição do verde (-a*) para vermelho (+a*) e b* representa a transição de azul (-b*) para amarelo (+b*). As variações totais de cores foram avaliadas pelo parâmetro ΔE*, calculado de acordo com a equação abaixo:

$$\Delta E^* = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$$

Análises microbiais

As contagens de placas microbianas foram determinadas de acordo com os padrões ISO para análises microbianas de alimentos, compiladas por Stoops et al. (2016) e Vandeweyer, 2017 para as contagens viáveis totais (TVC), *Enterobacteriaceae*, LAB, fungos e leveduras, e os endosporos bacterianos. Quanto às contagens de *E. coli* foram determinadas após incubação aeróbica em TBX (Tryptone Bile X-Glucuronide) por 24 horas a 44 ° C, as contagens de *B. cereus* foram determinadas após incubação aeróbica em ágar MYP (Mannitol Yolk Polymyxin) (30 ° C, 24 he 48 h), e *Salmonella sp*. as contagens foram determinadas pelo método de detecção de *Salmonella sp.* em alimentos, de acordo com a norma ISO 6579.

Teste de desafio com *E. coli*

Para a avaliação da eficácia dos processos de CVD contra *E. coli,* as cepas substitutas de *E. coli* ATCC 8739 e ATCC 25922 foram reunidas e inoculadas na alimentação das larvas antes do tratamento. O farelo de trigo foi inoculado com o conjunto de cepas de *E. coli*. YM vivos frescos foram então colocados no farelo contaminado (YMB) para investigar a ocorrência de contaminação por *E. coli* juntamente com TVC e *Enterobacteriaceae* em 24 horas e em 7 dias.

Resultados e Discussão

Amostras frescas de YM utilizadas neste estudo apresentaram conteúdo inicial de umidade (X_dbo) variando de 1,5316 ± 0,0367 a 1,8099 ± 0,0071 gg-1 (base seca, db) e atividade de água (aw0) variando de 0,980 ± 0,003 a 0,988 ± 0,000. As amostras branqueadas apresentaram X_dbo variando de 1,4562 ± 0,0540 a 1,9044 ± 0075 gg-1 (db) e aw0 variando de 0,986 ± 0,009 a 0,990 ± 001. Após os processos de secagem, o teor final de umidade X_dbf e a atividade da água a_wf foram reduzidos para valores iguais ou inferiores a 0,0315 g g-1 e 0,287 g g-1, respectivamente. Assim, esses YM secos são muito menos ou não expostos a bactérias e leveduras e crescimento de fungos, oxidação lipídica, escurecimento não enzimático (BELITZ, H. D., GROSCH, W., & SCHIEBERLE, 2009).

Neste estudo, uma vantagem do branqueamento YM antes da secagem foi sua influência na redução dos tempos de secagem. Foi notado que as amostras branqueadas atingiram a faixa alvo de atividade da água em tempos mais curtos (cerca de 30 min a menos) e temperaturas mais baixas do que as lavadas. Uma hipótese para esse comportamento é que o branqueamento facilitou a remoção de água das células YM durante a secagem. Também foi notado que para atingir a atividade da água entre 0,3-0,2, foi necessário aplicar pelo

menos 13 pulsos de vácuo para secagem de DVD, enquanto que para a KMFD, foram necessários 5 ciclos de aquecimento a vácuo. Todos os experimentos de secagem foram realizados em triplicata e mostraram boa reprodutibilidade.

A utilização da secagem a vácuo garante não apenas um ambiente de secagem com baixa concentração de oxigênio, mas também a diminuição da temperatura de saturação da água que leva a um aumento na taxa de evaporação sem atingir temperaturas muito altas, o que, por sua vez, contribui para reduzir as perdas sensoriais e nutricionais (ALIBAS, 2007). A exposição a altas temperaturas e em longos tempos de processamento pode não apenas degradar alguns nutrientes sensíveis ao calor, mas também favorecer reações de escurecimento e colapso dos tecidos que, por sua vez, levam ao escurecimento e ao encolhimento do YM (PURSCHKE, BRÜGGEN, SCHEIBELBERGER & JÄGER, 2017). As temperaturas e os tempos de processamento neste estudo foram relativamente menores do que os usados para outros estudos de secagem. A secagem do forno e do leito fluidizado, por exemplo, resultou em tempos de secagem mais longos (7 a 24 h) (PURSCHKE, B., BRÜGGEN, H., SCHEIBELBERGER, R., & JÄGER, 2017). No estudo de Kröncke et al. (2018), foi necessária uma temperatura de 130 ° C para secar YM a um a_w de 0,56 com o processo de secagem em leito fluidizado.

Com exceção do LAB, bolores e levedura e endosporos bacterianos aeróbicos, a faixa de todas as contagens (patógenos incluídos) está de acordo com os resultados obtidos por Caparros Megido et al. (2018), Klunder et al. (2012), Vandeweyer et al. (2017), Stoops et al. (2016) e Wynants et al. (2017). Diferenças entre LAB, levedura e bolores, e contagens de endosporos bacterianos aeróbicos deste estudo e outros podem ser o resultado da YM ser submetida a diferentes práticas de alimentação e/ou criação, já que os parâmetros microbiológicos da YM dependem dessas variáveis (VANDEWEYER et al. , 2017).

Os efeitos das combinações de enxágue mais secagem e branqueamento mais secagem na microbiota de larvas YM foram investigados. Para as amostras enxaguadas, notou-se que a técnica de secagem que reduziu todas as cargas microbianas. a maioria era o DVD; isso pode ser devido ao maior número de pulsos de vácuo aplicados durante a secagem. O KMFD reduziu consideravelmente todas as cargas microbianas, mas não conseguiu reduzir o LAB e as leveduras e moldou as cargas para o mesmo nível do DVD, talvez por ter menos pulsos de vácuo no processo.
O mesmo pode ser observado com as CVD, que não apresentaram pulso de vácuo na secagem, resultando nas menores cargas redutoras de LAB e leveduras e bolores. Os pulsos de vácuo demonstraram um efeito significativo e positivo na desativação dos microrganismos de YM durante o processo de secagem.

Para as amostras branqueadas, todas as três técnicas de secagem foram capazes de reduzir as cargas microbianas iniciais para <2 log ufc/g. A partir desses resultados, pode-se interpretar que, independentemente de qual dessas técnicas usadas, se YM forem branqueadas e secas nas mesmas condições (tempo, temperatura, pressão e higiene), suas cargas microbianas iniciais serão reduzidas a pelo menos <2 log ufc/g e não haverá potencialmente presença de *Salmonella spp.* e *E. coli* em 25 g das amostras. E pela mesma lógica, o branqueamento antes da secagem é primordial para garantir uma redução satisfatória das cargas microbianas YM. Neste estudo, a combinação de branqueamento e secagem (CVD, DVD ou KMFD) foi mais eficiente na redução da carga microbiana YM inicial do que na lavagem e secagem. Somente o branqueamento já poderia reduzir a carga microbiana e, provavelmente, exercer estresse na parede celular microbiana, desabilitando mais facilmente os microrganismos durante a secagem.

Antes da contaminação com a *E. coli,* só foi notado cargas de TVC e *Enterobacteriaceae.* Após a contaminação, a carga de *E. coli* no farelo contaminado e no YM aumentou após um dia, mas diminuiu no 7º dia em um nível mais baixo de contaminação, provavelmente devido ao aumento de *Enterobacteriaceae.* Alguns dos microrganismos dessa família podem ter suprimido o crescimento e/ou causado a diminuição de *E. coli* por uma possível competição. Outra explicação para isso pode ser o fato de a YM ser de sangue frio (BELL e KYRIAKIDES, 1998). Pelo mecanismo de uma resposta imune geral, os invertebrados são capazes de aumentar a resistência a todos os tipos de patógenos (RÂBERG et al. 1998). O gene do homólogo dorsal (TmDorX2) identificado no YM, que pode ser considerado um regulador positivo para a produção de peptídeos antimicrobianos contra E. coli no intestino, corpo gordo e hemócitos do YM jovem em resposta à infecção bacteriana e fúngica, conforme descrito por Keshavarz et al. (2019), pode ser outra explicação potencial para essa diminuição.

A combinação de branqueamento e CVD apresentou eficácia na redução das cargas de YM de TVC, *Enterobacteriaceae* e *E. coli* após o teste de desafio de *E. coli,* proporcionando resultados satisfatórios de

redução. A técnica de CVD pode fornecer um nível de letalidade em 5 D. As contagens YM secas a branqueamento para TVC e *Enterobacteriaceae* foram todas inferiores a 2,00 log cfu/g para todas as amostras. O uso de CVD, conforme estipulado neste estudo, garante a segurança da YM contra *E. coli*. Não apenas reduz a carga de *E. coli*, mas também evita ou minimiza o crescimento desse patógeno em YM, diminuindo a atividade da água.

Os resultados de cor são apresentados na tabela a seguir

Resultados de cor das YM frescas e secas

	L*	a*	b*	ΔE
Fresco	$40.10 \pm 0.46^{a**}$	6.51 ± 0.28^{ab}	31.08 ± 1.03^{a}	
CVD-R	36.83 ± 0.33^{d}	7.21 ± 0.30^{b}	20.29 ± 0.38^{b}	11.29 ± 0.65^{a}
CVD-B	38.65 ± 0.53^{b}	5.66 ± 0.23^{a}	15.82 ± 0.77^{c}	15.34 ± 0.27^{bc}
DVD-R	35.10 ± 0.17^{e}	6.51 ± 0.41^{ab}	17.30 ± 0.77^{c}	14.65 ± 0.48^{abc}
DVD-B	36.27 ± 0.18^{d}	7.05 ± 0.95^{ab}	16.32 ± 1.37^{c}	15.26 ± 0.80^{bc}
KMFD-R	31.01 ± 0.26^{c}	7.31 ± 0.30^{b}	21.96 ± 1.15^{b}	12.89 ± 0.24^{ac}
KMFD-B	34.52 ± 0.27^{e}	7.21 ± 0.81^{b}	15.01 ± 0.58^{c}	17.02 ± 0.71^{b}

O uso de qualquer dessas técnicas de secagem para secar as YM acarreta à mudança de cor. Foi notado que, para todas as técnicas de secagem, o brilho das YM diminuiu após o processo. Segundo Caivano e Del Pilar Buera (2012), a água dos tecidos frescos apresenta um índice de refração diferente do ar dos tecidos secos; a redução de água (umidade) durante a secagem pode ter afetado o brilho (L*), fazendo com que as YM secas pareçam menos brilhantes que as frescas. A vermelhidão da YM não apresentou variações significativas entre os pré-tratamentos e as técnicas de secagem. O amarelecimento das YM diminuiu após a secagem e, para esse parâmetro, os valores das YM branqueadas diminuíram mais do que os das enxaguadas. Pode-se interpretar que o branqueamento deve ter um efeito maior da lixiviação dos carotenóides das YM do que o enxágüe e pode ser a causa da redução do amarelecimento. Para as mudanças totais de cor (ΔE), os valores das YM branqueadas secas foram superiores aos das secas enxaguadas para todas as técnicas de secagem, a mesma faixa de ΔE foi observada por Kröncke et al. (2019), que secou as YM por secagem em estufa e liofilização.

Considerações Finais

A secagem condutiva a vácuo (CVD), a secagem condutiva multi-flash I (KMFD) e a secagem condutiva multi-flash II (DVD) podem produzir larvas de *Tenebrio molitor* (YM) secas com baixo teor de umidade e atividade da água em curtos tempos de secagem. Essas três técnicas, acopladas ao branqueamento YM, podem ser usadas para a redução das cargas microbianas iniciais de YM. YM podem ser intencionalmente contaminados com *E. coli* em um teste de desafio e a combinação de branqueamento mais CVD é eficaz na redução das cargas de *E. coli*. Alterações totais de cor mais altas são provocadas pelo branqueamento antes da secagem, mas como compensação, preserva mais a luminosidade inicial. Do ponto de vista dos tempos de secagem, a redução microbiana e a mudança de cor, CVD, DVD e KMFD podem ser alternativas às técnicas de secagem por congelamento, forno ou leito fluidizado para YM. Em geral, um branqueamento de apenas 15 segundos da YM antes da secagem leva a tempos de secagem mais curtos, maior brilho e redução microbiana mais significativa, tornando a YM microbiologicamente segura para a produção de alimentos e rações. As técnicas de secagem usadas nesse estudo poderiam ser usadas em estudos futuros, utilizando condições diferentes de pressão e temperatura, por exemplo.

Palavras-chave: Insetos comestíveis. Entomofagia. Larvas. Secagem a vácuo. Segurança de alimentos. Teste desafio microbiológico.

ABSTRACT

Larvae of *Tenebrio molitor* (YM) are incessantly being pointed as an alternative to conventional sources of animal protein, thanks to their nutritional value and low environmental impact for production. Notwithstanding, some studies have reported high water activity and different levels of microbial contamination. In that context, blanching and drying are generally employed to respectively reduce microbial loads and enzymatic activity and decrease the moisture content and water activity to conserve YM for long periods. This study aimed to dry rinsed (non-blanched) and blanched YM by conductive vacuum drying (CVD), conductive multi-flash drying I (KMFD) and conductive multi-flash drying II (DVD) to enable its use for food and feed. The effects on the microbiota and final coloration of YM were also investigated, as well as kinetic curves were performed. A challenge test (artificial contamination) was performed with *E.coli* by subjecting YM in a contaminated feed (wheat bran) with *E.coli* then to one of the drying techniques to evaluate its effectiveness on the decontamination of the pathogen. It was possible to reduce the water activity to 0.3 - 0.2 for all the samples. KMFD and DVD times were shorter than CVD, and for each technique, the times used for blanched samples to reach the targeted water activity were shorter than that for the rinsed samples. For the blanched samples, all the techniques showed satisfying microbiological reduction. For the rinsed samples, the same level of reduction was observed for DVD, for KMFD, except LAB and yeast and molds (2.5 and 2.3 log cfu/g, respectively), and for CVD, except TVC, LAB and yeast and molds (2.0, 4.6 and 2.7 log cfu/g, respectively). Through the challenge test (artificial contamination), it was possible to verify the effectiveness of the combination of blanching and conductive vacuum drying (CVD) in the reduction of YM *E.coli* load. The drying technique that preserved brightness the most was CVD. It was concluded that CVD, DVD, and KMFD can be used to dry YM. Moreover, blanching YM before drying leads to better results in brightness, microbial reduction and drying times.

Keywords: Edible insects. Entomophagy. Yellow mealworms. Vacuum drying. Food safety. Food security. Microbial challenge test.

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LISTA DE ABREVIATURAS E SIGLAS

ANOVA – Analysis of variance

AOAC – Association of Official Analytical Chemists

Blanched-CVD – Blanched larvae dried by vacuum drying

Blanched-DVD – Blanched larvae dried by conductive multi-flash drying II

Blanched-KMFD – Blanched larvae dried by conductive multi-flash drying I.

CMFD – Convective multi-flash drying

EFSA - European Food Safety Authority

FAO – Food and Agricultural Organization

KMFD – Conductive multi-flash drying I (vacuum pulses at 60 °C)

KMFDVD – Conductive multi-flash drying with 1 vacuum pulse

MFD – Multi-flash drying

MWMFD – Microwave multi-flash drying

PROFI – Laboratory of Physical Properties of Food

Rinsed-CVD – Non-blanched larvae dried by vacuum drying

Rinsed-DVD – Non-blanched larvae dried by Conductive multi-flash drying II

Rinsed-KMFD – Non-blanched larvae dried by Conductive multi-flash drying I

UFSC – Federal University of Santa Catarina

CVD - Vacuum drying with only one vacuum pulse applied at the beginning

YM – Larvae of *Tenebrio molitor*

YMB – YM submitted to the *E.coli* contaminated wheat bran

DVD – Conductive multi-flash drying II (vacuum pulse applied right at the beginning of the drying process and at every 10 minutes)

LIST OF SYMBOLS

Symbol	Description	Unit
a_w	Water activity	-
C_p	Specific heat	$Jkg^{-1}°C^{-1}$
dX/dt	Drying rate	-
m_{H2O}	Mass of water	g
m_p	Initial product mass	kg
m_s	Sample mass	g
m_{ss}	Mass of dry solids	kg
P	Pressure	mbar
t	Time	min
T	Temperature	°C
UR	Relative humidity	g 100 g^{-1}
X_{bs}	Dry base moisture	g g^{-1}
X_{bu}	Moisture on a wet basis	g g^{-1}
ΔH_v	Specific enthalpy of water vaporization	J kg^{-1}
Δm_w	Weight loss	kg
ΔT	Temperature variation	°C

SUMMARY

1 INTROCTION

The world's food demand has been growing significantly, driven by population increase and economic growth. With the depletion of natural resources (land, water, and energy), it is necessary to rethink our eating patterns and habits, particularly those related to meat consumption (VAN HUIS, VAN GURP, DICKE, TAKKEN-KAMINKER, & BLUMENFELD-SCHAAP,c 2014). For each kilogram of high-quality animal protein produced, the cattle are fed with approximately 6 kg of plant protein. A high amount of animal protein produced results in increased greenhouse gas emissions, deforestation, and environmental degradation (PIMENTEL & PIMENTEL, 2003; VAN HUIS et al., 2014).

Alternative sources of protein that moderate the impact of animal farming are plants with a high content of proteins, cultured meat from stem cells, seaweed, fungi, and insects (BHAT & FAYAZ, 2011; FLEURENCE, 1999; VAN HUIS et al., 2014). The habit of eating insects as food (entomophagy) has been considered as one of the most suitable solutions to guarantee global food security (ALEXANDRATOS & BRUINSMA, 2012; BELLUCO et al., 2013; McGREW, 2014; RAMOS-ELORDUY, GONZÁLEZ, HERNÁNDEZ, & PINO, 2002; VANTOMME, 2015).

According to Van Huis et al. (2014), insects have advantages compared to livestock rearing once they can reproduce fast, have a higher feed conversion efficiency (the ability of converting feed to body mass), require less water per kg of protein produced, and can be feed with organic wastes, reducing environmental impact. Also, insects present a low risk of transmission of zoonotic infections and emit relatively few greenhouse gases and ammonia (VAN HUIS et al., 2013).

Currently, more than 2000 insect species documented in the literature as edible insects are consumed by two billion people worldwide, and insect-based foods have recently become available in United States and some European countries (BELLUCO et al., 2013; JONGEMA, 2015; RAMOS-ELORDUY, 2008; SHELOMI, 2015). Among the edible insects, *Tenebrio molitor*, also called yellow mealworm (YM), are one of the most produced insects for food and feed due to their flexibility in livestock management, environmental sustainability, and attractive nutritional quality (BARSICS et al., 2017; CAPARROS MEGIDO et al., 2016; CORTES ORTIZ et al., 2016; RUMPOLD & SCHLÜTER, 2013b; VELDKAMP, VAN HUIS, & LAKEMOND, 2012).

The composition in nutrients of YM makes them an interesting source of functional proteins. Consequently, YM larvae have been evaluated for pet feeding, fishing bait, and human consumption. They are sold in multiple forms in some countries in North America and Europe (ADÁMKOVÁ, KOUŘIMSKÁ, BORKOVCOVÁ, KULMA, & MLČEK, 2016; CAPARROS MEGIDO et al., 2016, 2014; CORTES ORTIZ et al., 2016; DE MARCO et al., 2015).

Nevertheless, larvae of YM can present different levels of microbial contamination, encompassing potential human pathogens and spoilage microbes. Some studies reported the presence

of *Enterobacteriaceae*, lactic acid bacteria (LAB), mesophilic aerobes, spore-forming bacteria, psychrotrophic aerobic bacteria, aerobic bacterial endospores, yeasts and moulds (KLUNDER et al., 2012; RUMPOLD et al., 2014; STOOPS et al., 2016; VANDEWEYER et al., 2017a). Moreover, fresh larvae of YM present a moisture content up to 68% and high water activity (a_w 0,98) that highly expose them to microbiological spoilage, enzymatic and non-enzymatic (Maillard reaction) degradation (LEDL; SCHLEICHER, 1990; RAHMAN, 2007; TAOUKIS; RICHARDSON, 2007). Plus, YM generally can present up to 38% of fats, which exposes them to lipid oxidation (GHOSH et al., 2017; LENAERTS et al., 2018; ZHENG et al., 2013). Processing and storing YM for food or feed usually require a beforehand decontamination to extend its shelf life. Some studies have shown the importance of a preliminary heating step, such as blanching, which is also used to reduce the number of microorganisms in insects before drying (KLUNDER et al., 2012; RUMPOLD et al., 2014; STOOPS et al., 2016; VANDEWEYER et al., 2017a).

Drying is widely used as a preservation technique once it reduces the moisture content and a_w of foods, inhibiting the growth of microorganisms, reducing enzymatic activity, and chemical reactions (GEANKOPLIS, 1998; LABUZA, 1980). Consequently, it prolongs the shelf life of food products in addition to reducing storage and transportation costs (ALIBAS, 2007; RATTI, 2001). Kröncke et al. (2019) suggested that it could be more advantageous to find more sustainable and potentially cheaper drying methods for YM. For foods that can suffer damages or even losses of vitamins if exposed to high temperatures, the vacuum drying is one of the techniques widely used. It increases the rate of evaporation due to the decrease in the saturation temperature of the water. The maintenance of low temperatures is essential for thermo-sensitive products, in addition to establishing a drying environment with low concentrations of oxygen, contributing to reduce the sensorial and nutritional losses of the dehydrated products (ALIBAS, 2007; REIS, 2014).

In this context, conductive vacuum and multi-flash dryings are worth to be investigated in the dryings of YM. Multi-flash drying is a drying process that has been used in fruits and vegetable dehydration. In that technique, the product is heated at atmospheric pressure up to the desired temperature, and then a vacuum pulse is applied, leading to flash evaporation and, consequently, to the sample cooling (LAURINDO, PORCIUNCULA, ZOTARELLI, 2011). This drying technology allows producing dried food with low moisture content and low a_w in reduced processing times (LAURINDO, PORCIUNCULA, ZOTARELLI, 2011; ZOTARELLI; PORCIUNCULA; LAURINDO, 2012).

1.1OBJECTIVES

1.1.1 Main objective

This work aims to evaluate the influence of conductive vacuum drying (CVD), conductive multi-flash drying I (KMFD) and conductive multi-flash drying II (DVD) on the color and microbiota of *Tenebrio molitor* larvae.

1.1.2 Specific objectives

a) Determine the drying curves of rinsed and blanched *T. molitor* larvae dried by DVD and KMFD.

b) Evaluate the effects of CVD, DVD, and KMFD on microbiota and color of rinsed and blanched *T. molitor* with aw = 0.3 – 0.*2*.

c) Subject YM to a challenge test (artificial contamination) in a contaminated feed (wheat bran) with *Escherichia coli*, then to the drying treatment (CVD), and evaluate its effectiveness on the decontamination of the pathogen.

2 **LITERATURE REVIEW**

2.1 WORLD INCREASING POPULATION AND ITS CONSEQUENCES ON DIET BEHAVIOURS

The world's population has been increasing rapidly in the last years. Between 1950 and 2000, it moved from 2.7 to 6.7 billion people. Since then, the world has added approximately one billion inhabitants over the last twelve years, and, according to the results of the 2017 Revision, the world's population numbered nearly 7.6 billion as of mid-2017 (UNITED NATIONS, 2017).

Today, the world's population continues to grow, although more slowly than in the recent past years, it is growing by 1.10 percent per year, yielding an additional 83 million people annually. It is projected to rise by slightly more than one billion people over the next 13 years, reaching 8.6 billion in 2030, and increasing further to 9.8 billion in 2050 and 11.2 billion by 2100, as presented in Table 1 (UNITED NATIONS, 2017).

The rising world population is attached to the growth in the economy, urbanization, and, above all, the increase in food demand (quantity and quality), which arise from vegetable to animal-based diet. People not only need more food but also need adequate and high-quality food, which implies that food production will have to be almost the double of the current production to be able to feed everyone (VAN HUIS et al., 2013). According to Alexandratos and Bruinsma (2012) and Pelletier and Tyedmers (2010), the demand for food of animal origin, for example, is projected to increase by between 70% and 75% to cope with the demand of additional 2 billion people, from 2012 to 2050.

Producing more food is coupled with using more resources, and the resources that are currently being used in food production are already under stress and are impacting negatively on the environment (GODFRAY et al., 2010; HALLSTRÖM; CARLSSON-KANYAMA; BÖRJESSON, 2015; KLUNDER et al., 2012). For that reason, the apparent limits to producing food for the rising global population have been a centre of preoccupations and discussions lately. It comes out that the meat sector is one of the leading polluters in the food industry with environmental impacts that influence three dimensions: climate change (global warming, acidification, and eutrophication potential), consumption of natural resources (above all land, water, and energy), and polluting the environment with various types of waste and wastewater discharge (ALEXANDRATOS; BRUINSMA, 2012; DJEKIC, 2015; VRIES; BOER, 2010). Therefore, an alternative to satisfying the food demand with fewer damages is necessary.

Table 1 - The population of the World in 2017, 2030, 2050, and 2100.

	Population (millions)			
Region	***2017***	***2030***	***2050***	***2100***
World	7550	8551	9772	11184
Africa	1256	1704	2528	4468
Asia	4504	4947	5257	4780
Europe	742	739	716	653
Latin America and the Caribbean	646	718	780	712
Northern America	361	395	435	499
Oceania	41	48	57	72

Source: Adapted from United Nations, 2017

2.2 ALTERNATIVE TO FEEDING THE INCREASING WORLD POPULATION: ENTOMOPHAGY

To be able to satisfy the rising food demand currently and in the future, there is a need to re-evaluate what we eat and, more importantly, how we produce what we eat. Suggested solutions stipulate to reduce meat consumption, intensify the efficiency of the food chain from the field to the final product ('field to fork'), or change diets towards food commodities necessitating less land (ALEXANDRATOS and BRUINSMA, 2012; PELLETIER and TYEDMERS, 2010).

One of the alternatives that have consistently been approved in recent years is insects. Entomophagy, the habit of eating insects as food, has been practiced by humans on every inhabited continent. Up until the present, although it's still a taboo and seen as unappetizing in certain societies, entomophagy is traditionally practiced by over 2 billion people in many Asian, Central American, Oceania, and African regions (MCGREW, 2014; RAMOS-ELORDUY, 2008; YEN, 2015).

Insects are a class of animals within the arthropod group that have a chitinous exoskeleton, a three-part body (head, thorax, and abdomen), three pairs of jointed legs, compound eyes and two antennae (VAN HUIS et al., 2013). They are among the most diverse groups of animals on the planet (more than 1 million described species). The total number of species is estimated at 6 -10 million, and the class potentially represents over 90 percent of the differing animal life forms on Earth (VAN HUIS et al., 2013).

At present, nearly 2000 species of insects, collected from agricultural fields or even from farms, freshwater ecosystems, forests, and deserts, are known to be edible for human consumption. They have served as a resource of food in many different cultures and gastronomies of some contemporary cultures since prehistory. Nowadays, insects are re-discovered as one instrument to offer nutrients, play a significant and variable role in diets, and constitute a vital source of nutritious food for many (ADÁMKOVÁ et al., 2016; HANBOONSONG; JAMJANYA; DURST, 2013; ROTHMAN; RAUBENHEIMER; CHAPMAN, 2011).

The most commonly eaten insect groups are beetles, caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leaf and planthoppers, scale insects and true bugs, termites, dragonflies and flies (JONGEMA, 2015; VANTOMME, 2015; YEN, 2015).

Several factors are responsible for triggering the repugnance towards eating insects as food have been identified: from unique sensory properties and health safety issues as well as the reminders of something alive (ADÁMKOVÁ et al., 2016). As reported by Cardello (2003), food acceptance strongly depends both on intrinsic (sensory characteristics) and extrinsic factors such as cognitive, cultural, and social aspects. However, as pointed out by Van Huis et al. (2013), food preferences are not permanent and can change over time. Even though entomophagy has always been considered as "rural" and "barbarian" by Western society, recently there has been a growing consideration toward insect-based products in the US and Europe due to the increasing attention and conscientization from research institutes, food industry and legislators to the production process of these animals (CAPARROS MEGIDO et al., 2014).

2.3 WHY EATING INSECTS?

Insects are the most abundant multicellular organisms on planet Earth and are thought to account for more than 70% of all species. Insects are also among the most diverse groups of organisms in the history of life (VAN BROEKHOVEN et al., 2015; SCARAFFIA; MIESFELD, 2013).

More than 2 billion people around the world eat insects, generally as a whole in many cultures. But entomophagy does not have to be related only with ingesting the whole insects anymore. Many commercial food products are nowadays fortified to increase their nutritional value. Edible insects are prodigious material for food fortification for several reasons, being their high content in the protein of high biological value with a good amino acid profile and a high level of digestibility(OSIMANI et al., 2017; RUMPOLD; SCHLÜTER, 2013b).

Many species of insects are even richer in protein than beans (23.5% protein), lentils (26.7% protein), and soybeans (41.1% protein). And the protein digestibility of some of them has been reported to vary between 77 and 98% (ADÁMKOVÁ et al., 2016; ZIELIŃSKA et al., 2015).

Moreover, insects are a good source of a variety of micronutrients such as minerals (copper, iron, magnesium, manganese, phosphorus, selenium, and zinc), vitamins (riboflavin, pantothenic acid, biotin, and folic acid) and fiber (BELLUCO et al., 2013). Their lipid profile is good for humans; they are a source of unsaturated fatty acids, for example, omega-3 (ZIELIŃSKA et al., 2015).

Besides being qualified as a good source of nutrients, the production of edible insects in developing countries is supported by various institutions such as the Food and Agriculture Organization of the United Nations. They are also thought to be an economic trigger, as their

production not only offer some jobs; also their emerging industry was estimated to be worth $ 20 million in the last years, increasing to $ 360 million by 2020 in both North America and Europe (ZIELIŃSKA; KARAŚ; BARANIAK, 2018).

Overall, according to Van Huis et al. (2013), entomophagy is worth to be practice because:

- Insects are healthy, nutritious alternatives to conventional staples such as chicken, pork, beef, and even fish (from ocean catch).
- Insects promoted as food release considerably fewer greenhouse gases than most livestock (methane, for instance, is produced by only a few insect groups, such as termites and cockroaches).
- Insect rearing is not necessarily a land-based activity and does not require land-clearing to expand production. The feeding is the major requirement for land.
- The ammonia emissions associated with insect rearing are also far lower than those linked to mainstream livestock.
- Because they are cold-blooded, insects are very efficient at converting feed into protein (crickets, for example, need 12 times less feed than cattle, four times less feed than sheep, and half as much feed as pigs and broiler chickens to produce the same amount of protein).
- Mini livestock offer livelihood opportunities for both urban and rural people. Insect rearing can be low-tech or very sophisticated, depending on the level of investment.

A list of insect species that offer the greatest potential to be used as food and feed in the EU, proposed by The EFSA (2015), includes *Musca domestica, Hermetia illucens, Zophobas atratus, Alphitobius diaperinus, Galleria mellonella, Achroia grisella, Bombyx mori, Acheta domesticus, Gryllodes sigillatus, Locusta migratoria migratorioides, Schistocerca Americana and Tenebrio molitor*.

2.4 *TENEBRIO MOLITOR.*

Tenebrio molitor, also known as yellow mealworm (YM), is a species of the *Tenebrionidae* family, commonly known as darkling beetles. The YM biological life cycle (6-8 months) is divided into four distinct stages, which are egg, larva, pupa, and adult; for food and feed, they are used in larval stage (GHALY; ALKOAIK, 2009; KIM et al., 2015; LI; ZHAO; LIU, 2013; VAN BROEKHOVEN et al., 2015)

Females of YM lay an average of 400 - 500 ovoid and elongated eggs, covered with a waxy layer (GHALY; ALKOAIK, 2009). Eggs generally hatch to larvae after 4 days when around 26 – 30 ºC, but can be extended to weeks in lower temperatures. Then, it starts the larval stage, which has been reported to range from 75 to 90 days. The larva develops in an exoskeleton to support and protect its body, which color changes from white to brown over time (HARDOUIN; MAHOUX, 2003;

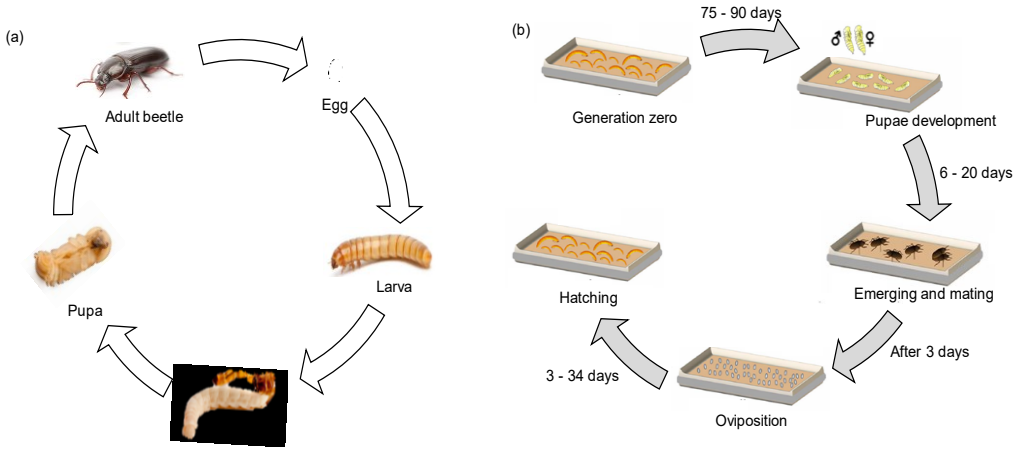
SPENCER, W. AND SPENCER, 2006). A light yellow-brown color characterizes the mature larva. After a few days, YM turns yellowish and produces a hard chitinous exoskeleton, leading to the pupae stage, reported to have a duration varying from 6 to 20 days (BAEK et al., 2015; BAJŽELJ et al., 2014; HILL, 2002; SIEMIANOWSKA et al., 2013). Afterward, the adult stage is characterized by a soft white exoskeleton that darkens over time, representing the final metamorphosis to reach the adult phase. The duration of the adult stage is reported to vary from 16 to 173 days, starting oviposition after only three days as an adult (DAMBORSKY, M. P., SANDRIGO-YBRAN, T. B. M. E., & OSCHEROV, 1999; MANOJLOVIC, 1987).

The fast-growing characteristic of YM allows its easy rearing in laboratories, as well as in large-scale industrialized systems, being fed with cereals or by-products of the food industry. During the larval stage, YM eats to store energy for reaching the pupal and adult stage. Larvae typically measure about 2.0 cm or more, whereas adults are generally between 1.25 and 1.8 cm in length (GHALY; ALKOAIK, 2009).

Usually, they are reared on a feeding substrate in plastic trays or boxes at an initial density of 5 larvae/cm2 (CORTES ORTIZ et al., 2016). For commercial purposes, larvae should be harvested at the pre-pupal stage in which they have the highest weight (SAUVANT, D. PEREZ, J.-M. TRAN, 2004).

The cycle life of *T. molitor* and the schematic drawing of YM rearing steps are illustrated in Figure 1.

Figure 1 - (a) The life cycle of *Tenebrio molitor* and (b) developing (molting) steps.



Source: Adapted from Osimani et al. (2018)

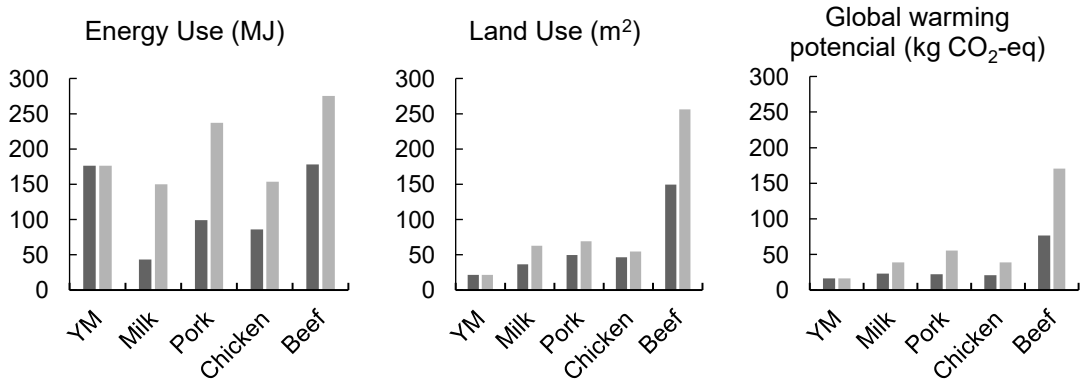
YM are one of the most produced insects for food and feed because they present advantages like environmental sustainability.

2.4.1 Environmental benefits

For each kilogram of high-quality animal protein produced, cattle are fed approximately 6 kg of vegetable protein, while YM can be fed wastes from wheat, corn, or fruits industry. (PIMENTEL; PIMENTEL, 2003). Also, the high consumption of animal protein increases the emission of gases that cause the greenhouse effect, as well as deforestation and degradation of the environment (VAN HUIS et al., 2014).

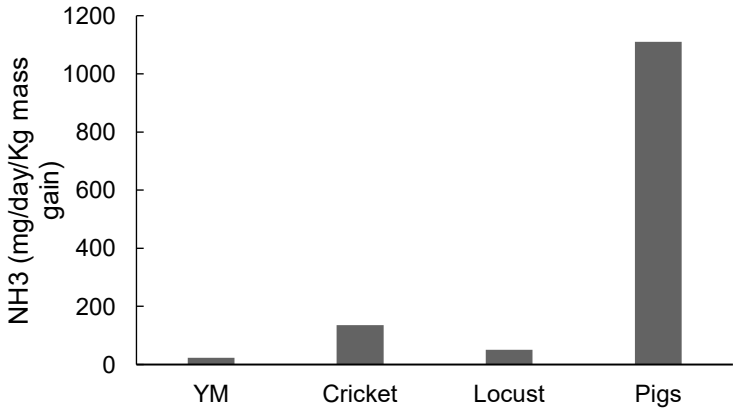
According to (OONINCX; DE BOER, 2012), YM is a more environmentally friendly source of animal protein. In terms of energy, land use, and global warming, for example, producing protein from YM is more likely than from beef, milk, and pork, as shown in Figure 2. Additionally, YM produces much less ammonia per kg of mass gain than do locusts, crickets, and pigs, as demonstrated in Figure 3. As for water footprint, the production of a ton of YM requires less water than the same quantity of pork and beef, as presented in Figure 4.

Figure 2 - Greenhouse gas production (global warming potential), energy, and land use from the production of 1 kg of protein from mealworms, milk, pork, chicken, and beef. The black bars are minimal values, and the grey bars are maximum values.



Source: Adapted from Oonincx & de Boer (2012)

Figure 3 - Production of ammonia per kg of mass gain for *Tenebrio molitor* (YM), cricket, locust, and pigs.



Source: Adapted from Oonincx et al. (2010)

Figure 4 - Water footprint per edible ton of mealworms and classic sources of animal protein.

Source: Adapted from Grau, Vilcinskas, & Joop (2017)

The energy used to produce one kg of fresh YM is similar to that used to produce the same amount of traditional livestock; however, comparing with beef, chicken, pork, and even milk, the land area required to produce one kg of fresh YM is much smaller. The production of greenhouse gases (CO_2, N_2O, and CH4) is significantly lower for mealworms compared with livestock and other insects like crickets and locusts (OONINCX et al., 2010; OONINCX; DE BOER, 2012). In addition to environmental benefits, YM present an attractive nutritional quality.

2.4.2 Nutritional value

Fresh YM larvae have up to 71% moisture (NOWAK, PERSIJN, RITTENSCHOBER & CHARRONDIERE, 2016). Among the dry solid content, YM presents a high content of protein (40 - 50%) and lipid (30 - 38%), including a high level of mono- and poly-unsaturated fatty acids (50.0% and 10.0%, respectively), 2% or more of fiber (mainly of chitin) and 1.55% of ash (ZHENG et al., 2013). Depending on the rearing substrate, the ratio of omega-6 to omega-3 (ω-6/ω-3 ratio) may be as low as 6.8 (FINKE, 2007; GRAU; VILCINSKAS; JOOP, 2017; MARONO et al., 2015; PAUL et al., 2017; SIEMIANOWSKA et al., 2013). Table 2 shows the fatty acid content of YM.

Table 2 - Fatty acids content of *Tenebrio molitor* (gram per 100 g of sample).

Fatty Acids	**Fresh**	**Dried**
Myristic acid (C14:0)	2.99	3.26
Palmitic acid (C16:0)	15.65	17.21
Stearic acid (C18:0)	2.86	3.06
Oleic acid (C18:ln9)	42.38	44.36
Linoleic acid (C18:2n6)	32.01	31.63
Linolenic acid (C18:3n3)	1.54	1.46
Eicosanoid acid (C20:ln9)	0.43	0.39
Arachidonic acid (C20:4n6)	0.44	0.50
Docosatetraenoic acid (C22:4n6)	0.54	0.41

Source: Adapted from Heidari-Parsa et al., 2018

Ramos-Elorduy et al. (2002) and Zhao et al. (2016) found that oils extracted from YM are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids; and the nutritional importance of these two essential fatty acids has been proven to enhance the healthy development of children (MICHALSEN et al., 2009).

Proteins from YM larvae contain essential amino acids in quantities that are necessary for humans (YI et al., 2013). Gould and Wolf (2018) stated that the protein extracted from mealworms could be used as an alternative to current protein-based emulsifiers in food formulations. Table 3 depicts the amino acid content of YM.

Table 3 - The amino acid content of *Tenebrio molitor* (grams per 100 g of protein).

Amino Acids	**Fresh**	**Dried**
Isoleucine (IIe)*	1.72	1.83
Leucine (Leu)*	3.02	3.13
Lysine (Lys)*	2.41	2.50
Methionine (Met)*	0.50	0.52
Phenylalanine (Phe)*	1.44	1.55
Threonine (Thr)*	1.60	1.70
Valine (Val)*	2.36	2.57
Histidine (His)*	1.17	1.38
Arginine (Arg)*	2.02	2.23
Threonine (Thr)*	1.45	1.70
Serine (Ser)	2.01	2.23

Source: Adapted from Heidari-Parsa et al., 2018. *indicate essential amino acids

Besides the fact that they are a good source of essential amino acids, niacin, pyridoxine, riboflavin, folate, and vitamin B12 (NOWAK et al., 2016; PAYNE et al., 2016), YM are known for their mineral content as presented in Table 4.

Table 4 - The mineral content of *Tenebrio molitor* (mg of mineral per kg of the sample).

Minerals	Fresh	Dried
Calcium (Ca)	514.12	500.12
Phosphorus (P)	950.12	976.36
Potassium (K)	932.63	953.20
Iron (Fe)	65.36	68.20
Magnesium (Mg)	1596.30	1630.14
Zinc (Zn)	96.14	106.31
Copper (Cu)	16.96	19.05
Manganese (Mn)	1.14	-
Sodium (Na)	133.16	-

Source: Adapted from Heidari-Parsa et al., 2018; Dobermann; Swift; Field, 2017 and Veldkamp et al., 2012).

Moreover, YM present good digestibility and are a potential protein alternative for animal meals, especially for the replacement of soybean. They have been reported as one of the most promising insects to be used as feed and food and can be found in many forms, such as canned, dried, or powdered. They are usually dried or boiled in water and have been used as feed in many experiments (BOVERA et al., 2015).

2.4.3 *Tenebrio molitor*-based products

YM presents excellent digestibility and is a potential protein alternative for animal meals, especially for the replacement of soybean (BOVERA et al., 2015). They have been reported as one of the most promising insects to be used as feed and food and can be found in many forms, such as canned, dried, or powdered. They are usually dried or boiled in water and have been used as feed in many experiments, as shown in Table 5.

Literature also reports YM as food for humans (AZZOLLINI et al., 2016; GRABOWSKI & KLEIN, 2017b, 2017a; PAYNE et al., 2016a, PAYNE, SCARBOROUGH, RAYNER, & NONAKA, 2016b; PAYNE, SCARBOROUGH, RAYNER, & NONAKA, 2016c). In some countries, where entomophagy is perceived as unpleasant by a significant amount of the population, YM is currently reared at great quantities and are now being included in human foods (CLAEYS, 2014).

Stoops et al. (2017) made a minced meat product from YM larvae for human consumption. Tortillas are enriched with YM in Mexico (AGUILAR-MIRANDA et al., 2002). Buqadilla (spicy Mexican leguminous food product made of chickpeas and YM at 40%) is a snack found in the Dutch market. In several restaurants and canteens where the product was tested, it got positive results for its taste and smooth structure (VAN HUIS et al., 2014).

Crikizz is a snack made with spicy- popped-insects and sold in Europe. It is based on YM and cassava, wherein its composition varies from 10% to 20% by the product line. Its texture has been reported to be as crunchy as other snacks, while its taste was very pleasing and different from other

snacks. The product even won a prize in the French national contest Eco-trophélia 2012 for culinary innovation (VAN HUIS et al., 2014).

Dried YM powder can be added into bread, flour, instant noodles, snacks, pastries, biscuits, candy, and condiments. They can also be consumed whole as meals and side dishes or processed into nutraceutical supplements to fortify the human body's immune system as observed in many countries (SEVERINI et al., 2018). In Brazil, YM-based food for human consumption, for instance, pizzas, pan-fried, crystallized YM, and cakes, have recently been prepared.

Nevertheless, larvae of YM can also present hazards like their microbial contamination (STOOPS et al., 2016).

2.4.4 Microbial of *Tenebrio molitor* larvae

According to Grau; Vilcinskas; Joop, (2017), YM is generally consumed without removing the gastrointestinal apparatus, and any of the microorganisms therein (including pathogens) can be transmitted to human consumers and livestock. The levels of microbiological contamination of YM depends on the environment they are grown. Many studies have reported that YM harbors a diverse microbial community, encompassing mainly potential spoilage microbes (KLUNDER et al., 2012; RUMPOLD et al., 2014; STOOPS et al., 2016; VANDEWEYER et al., 2017b). The presence of *Enterobacteriaceae*, LAB, mesophilic aerobes, spore-forming bacteria, psychrotrophic aerobic bacteria, aerobic bacterial endospores and yeasts and molds has been reported (OSIMANI et al., 2018; STOOPS et al., 2016).

Many studies reported the absence of *Escherichia coli*, *Salmonella spp*., *Campylobacter spp*., *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* in YM microbiota (CRIPPEN et al., 2012; EFSA, 2015; GIACCONE V, 2005; HEIDARI-PARSA et al., 2018; TEMPLETON et al., 2006); unlike *Listeria ivanovii* and *Penicillium spp.* that have already been encountered (GRABOWSKI; KLEIN, 2017b).

Moreover, fresh larvae of YM present a moisture content (up to 68%) and a_w (0.98) that make them susceptible not only to microbiological spoilage, enzymatic and non-enzymatic (Maillard reaction) degradation, but also to lipid oxidation (BONAZZI; DUMOULIN, 2014; GHOSH et al., 2017; GUSTAVO V. BARBOSA-CÁNOVAS, ANTHONY J. FONTANA JR., SHELLY J. SCHMIDT, 2007; LEDL; SCHLEICHER, 1990; RAHMAN, 2007; TAOUKIS; RICHARDSON, 2007).

Given their level of microbial contamination, processing YM for food or feed requires a beforehand decontamination.

Table 5 - Animals fed with *Tenebrio molitor*, procedure of feeding, and results.

	Animal	Procedure	Results and conclusions	Reference
1	Broiler chicken	YM larvae replacing soybean meal (SBM)	•No important influence on the growth performance and carcass characteristics, nor on chemical and physical properties of chicken meat; •The feed conversion was better for the YM group; •The weight of the spleen and the full digestive system were lower for the SBM group.	Bovera, Loponte, & Marono (2016)
2	Broiler chicken	Substituting 250 g/kg of the basal diet with YM	•The digestibility coefficients of all 17 amino acids analyzed were higher in the basal diet with YM; •YM is suitable to feed broilers as a valuable source of digestible amino acids.	De Marco et al. (2015)
3	Broiler chicken	YM were used as broilers feedstuff (0, 5, and 10%)	•No significant differences among treatments after 15 days; •YM has the potential to be used as a protein source for raising broilers; •YM transformed low-nutritive waste from the food industry into a high-protein feed.	Ramos-Elorduy, González, Hernández, & Pino (2002)
4	Broiler chicken	YM replaced soybean meal (SBM)	•Increased overall conversion ratio when YM was present; •SBM promoted a higher protein efficiency ratio, but the European efficiency factor was reduced when comparing to YM meals; •SBM favored albumin-to-globulin ratio, and was disadvantageous to the amount aspartate aminotransferase and alanine aminotransferase in the chicken; •Uric acid was lower in broilers fed with YM*;* •YM can completely replace soy meal in broiler diets during the growing period.	Bovera et al. (2015)

5	Rainbow trout (*Oncorhynchus mykiss*)	Rainbow trout fed with YM inclusion in 0%, 25%, and 50% of the meal weight basis	•The feed conversion ratio was significantly higher in 0% than in 25% and 50% while the opposite trend was observed for protein efficiency ratio and specific growth rate; •The survival rate was significantly lower in 0% than in 25% and 50%; •The apparent digestibility of protein was significantly lower in the 50% group.	Belforti et al. (2015)
6	Tilapia (*Oreochromis niloticus*)	YM larvae were used as a partial (25 and 50%) protein replacement in fishmeal (FM) and soy meal (SM) in fish diets	•YM inclusion reduced growth performance by 29% and affected the muscle fatty acid profile; •A partial replacement of FM and total replacement of SM with YM did not affect the feed intake, in vitro protein digestibility, and the amino acid composition of the muscle and the biometric indexes.	Sánchez-Muros et al. (2016)
7	Catfish (*Ameiurus melas*)	Fish were fed with fishmeal (FM) and YM	•Fishes fed with YM reached a final mean body weight significantly bigger than those in the FM group; •The survival rate of the FM group (79%) was higher than that of YM (70%).	Roncarati, Gasco, Parisi, & Terova (2015)
8	Pacific white shrimp (*Litopenaeus vannamei*)	YM replaced fishmeal partially and totally (0%, 25%, 50%, 75%, and 100%)	•Weight gain, specific growth rate, feed intake, feed conversion, survival and protein retention were not affected when YM replaced fishmeal; •No differences in protein content, but lipid content rose from 1.13% to 1.88% after YM replaced fishmeal.	Panini et al. (2017a, 2017b);
9	Blackspot seabream (*Pagellus bogaraveo*)	YM replaced fishmeal partially (0%, 25%, and 50%) for 131 days:	•The skin ventral region was redder in the 50% group; •Water holding capacity and texture characteristics (hardness, cohesiveness, resilience, gumminess, and adhesiveness) did not show significant differences;	Iaconisi et al. (2017)

			•The diet affected the fatty acids profile, whereas it did not affect proximate fillet composition.	
10	European sea bass (*Dicentrarchus labrax*) juveniles	Addition of a full-fat YM (0%, 25%, and 50%)	•Addition of YM altered the fatty acid profile of the whole fish; •50% inclusion led to a worsening of final body weight, weight gain, specific growth rate, and feeding rate; •Dietary inclusion of YM increased protein and structural carbohydrate digestibility.	Gasco et al. (2016)
11	African catfish	YM replaced fishmeal partially and totally (0%, 20%, 40%, 60%, 80%, and 100%)	•Growth and feed utilization efficiency of catfish fed up to 40% substitution of fish meal with YM were quite the same compared to fish fed the control diet; •Catfish fed from 20% to 80% substitution of the fish meal still displayed good growth and feed utilization efficiency; •Catfish fed only with YM showed a slight depression in growth performance, but combining YM and catfish pellets led to better growth than fish fed with the commercial catfish pellet only; •Catfish fed with YM diets tended to have significantly higher lipids in their carcass.	Ng, Liew, Ang, & Wong (2001)
12	Gilthead seabream juveniles (*Sparus aurata*)	YM replaced fishmeal partially (0%, 25%, and 50%).	•Up to 25%, the inclusion of YM did not lead to adverse effects on weight gain and final weight, while a slight depression was observed for protein efficiency ratio and feed conversion ratio; •50% of YM induced growth reduction and had less favorable outcomes for specific growth rate, feed conversion ratio, and protein efficiency ratio; •No differences for the whole-body proximate composition was observed; •Substitution of fish meal protein in diets for gilthead seabream juveniles is viable up to 25% without adverse effects on growth performance and whole-body proximate composition.	Piccolo et al. (2014)

2.4.5 Decontamination of *Tenebrio molitor* larvae

Different techniques (starvation, blanching, cooking, drying, high hydrostatic pressure, and direct and indirect plasma treatments) have been applied to decontaminate and preserve YM larvae. The results are presented in Table 6.

Starvation of YM larvae is a commonly included procedure at the end of the larval rearing process, especially when aiming for human consumption. It is often presumed that these practices reduce the microbial contamination of edible insects by cleaning out the insect's gastrointestinal apparatus. However, Wynants et al. (2017) reported that the differences in the microorganisms count among the larvae that were deprived of food for 48 h were not significant.

Boiling and vacuum-cooking were the most efficient techniques to reduce the microbial load of YM while maintaining high levels of digestible protein and polyunsaturated fatty acids. On the other hand, frying effectively reduced the microbial load but produced YM with very high lipid contents (CAPARROS et al., 2018). Some studies postulate that a heating step, such as blanching, is necessary to reduce microbial count on insects before they are placed on the market (VANDEWEYER et al., 2017a).

Rumpold et al. (2014) observed that the most efficient treatments to reduce the overall microbial count was achieved by heat treatments at 90 °C (drying and vacuum cooking), while direct and indirect plasma treatments were not efficient for decontamination. Overall, some authors that have investigated the microbial load of YM and the forms of decontamination are presented in Table 6. After microbial decontamination, preservation technique like drying is needed to lower their high water activity to avoid the microbial growth (LENAERTS et al., 2018).

Table 6 - Microbiota of fresh YM larvae before and after different treatments.

S.No	Product	Procedures	M.O	Microbiota (log cfu/g)			References
1	Fresh	Insects were sedated by cooling (±4 °C, 1 h)	Total viable aerobic count	8.4 ± 0.1			Vandeweyer, Crauwels, Lievens, & Van Campenhout (2017)
			Lactic acid bacteria	7.7 ± 0.1			
			Enterobacteriaceae	7.4 ± 0.1			
			Aerobic bacterial endospores	3.1 ± 0.3			
			Psychrotrophic aerobic count	6.6 ± 0.4			
			Yeasts and molds	5.3 ± 0.3			
			Salmonella spp.	Absence in 25g			
			Listeria monocytogenes	Absence in 25g			
2	Fresh	The pilot production chain of fresh *Tenebrio molitor* larvae was investigated		**Batch 1**	**Batch 2**		Osimani et al. (2018)
			Lactic acid bacteria	7.7 ± 0.1	8.2 ± 0.3		
			Enterobacteriaceae	6.1 ± 0.1	7.1 ± 0.2		
			Total mesophilic aerobes	5.5 ± 0.2	8.7 ± 0.1		
			Spore forming bacteria	3.0 ± 0.1	3.7 ± 0.2		
			Salmonella spp.	Absence in 25g	Absence in 25g		
			Listeria monocytogenes	Absence in 25g	Absence in 25g		
	Dried, powder, and parfait	Freeze-dried, Powder freeze-dried and Parfait contending *Tenebrio molitor*		**Dried**	**Powder**	**Parfait**	Grabowski & Klein (2017b)
			Aerobic bacterial count	<6.7;	<6.7;	>6.7;	
			Aerobic bacterial count (after cooling)	<4.0;	<4.0;	>4.0;	
			Aerobic bacterial count (at the counter)	<6.0;	<6.0;	>6.0;	
			Enterobacteriaceae (after cooling)	<2.0;	<2.0;	>2.0;	
			Enterobacteriaceae (at the counter)	<3.0;	<3.0;	>3.0;	
			Escherichia coli	>1.0;	>1.0;	>1.0;	
			Coagulase-positive *staphylococci*	>3.0;	>3.0;	>3.0;	
			Listeria ivanovii	Presence	-	-	
			Penicillium spp.	Presence	-	-	

4	Fresh, blanched, chilled and microwave dried	Blanched in boiling water (10, 20 or 40 s) ↓ Chilled water (60 s) ↓ Sieve (30 s) ↓ Sealed and kept for 6 days in chilled storage (3.7 °C ± 1.7 °C) and Blanched in boiling water (40 s) ↓ Chilled water (60 s) ↓ Sieve (30 s) ↓ Microwave drying		**Control Batch (10 s)**	**Control Batch (20 s)**	**Control Batch (40 s)**	**Blanched - BL (10 s)**	**BL (20 s)**	**BL (40 s)**	**Chilled storage (BL-10 s)**	**Chilled storage (BL-20 s)**	**Chilled storage (BL-40 s)**	**Not BL only drying (10 min)**	**Drying (8 min)**	**Drying (10 min)**	**Drying (13 min)**	**Drying (16 min)**	**Drying (20 min)**	Vandeweyer, Lenaerts, Callens, & Van Campenhout (2017)
			Total viable aerobic count	7.9 ± 0.3	8.2 ± 0.7	7.6 ± 0.4	3.5 ± 0.8	1.8 ± 0.4	2.0 ± 0.4	2.7 ± 0.2	2.9 ± 0.2	3.5 ± 0.3	2.9 ± 0.7	3.4 ± 0.8	3.3 ± 1.3	2.1 ± 0.4	1.3 ± 0.2	1.3 ± 0.2	
			Lactic acid bacteria	2.6 ± 0.3	2.8 ± 0.4	3.1 ± 0.1	2.8 ± 1.0	4.7 ± 0.7	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	1.4 ± 1.1	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.3	1.7 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	
			Enterobacteriaceae	7.3 ± 0.5	7.5 ± 0.6	7.1 ± 0.9	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	1.2 ± 0.3	1.1 ± 0.1	2.1 ± 0.1	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	
			Aerobic bacterial endospores	7.4 ± 0.2	7.0 ± 0.1	6.9 ± 0.2	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	1.3 ± 0.4	<1.0 ± 0.0	1.3 ± 0.1	2.3 ± 0.5	1.8 ± 0.8	2.0 ± 1.5	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	
			Psychrotrophic aerobic count	3.8 ± 0.5	3.5 ± 0.2	3.5 ± 0.4	<2.0 ± 0.0	<2.0 ± 0.0	1.5 ± 0.8	<2.0 ± 0.0	<2.0 ± 0.0	<2.0 ± 0.0	<2.0 ± 0.0	2.2 ± 0.4	<2.0 ± 0.0	<2.0 ± 0.0	<2.0 ± 0.0	<2.1 ± 0.1	
			Yeasts and molds	7.2 ± 0.4	6.0 ± 0.2	6.5 ± 0.6	<1.0 ± 0.0	<1.0 ± 0.0	<1.0 ± 0.0	1.1 ± 0.1	1.3 ± 0.1	2.8 ± 0.8							

5	Oven-cooked, vacuum-cooked, fried and boiled	Fresh mealworms ↓ Fasted 24 h to reduce the gastrointestinal ↓ Frozen (-18°C) ↓ Cooked methods	**Vacuum cooking:** plastic bag immersed in water (74 °C for 60 min) **Frying:** pan-fried (1 min in 15.0 ml olive oil) **Boiling**: Hot water (100 °C for 1 min) **Oven cooking**: oven (70 °C for 15 or 30 min)		**Fresh**	**Oven-cooked (15 min)**	**Oven-cooked (30 min)**	**Vacuum -cooked**	**Fried**	**Boiled**	
				Total aerobic count	8.5 ± 0.1	6.7 ± 0.1	6.1 ± 0.1	3.9 ± 0.7	3.3 ± 0.1	1.6 ± 0.8	Caparros Megido et al. (2018)

6	Direct and indirect plasma treatment, high hydrostatic pressure treatment and thermal treatments	Fresh mealworms ↓ Frozen (-18°C) ↓ Treatments	**Vacuum cooking:** plastic bag immersed in water (45 °C for 5, 10 and 15 min and 90 °C for 2.5, 5, 10 and 15 min) **Drying:** oven 90°C for 2.5, 5, 10 and 15 min **High hydrostatic pressure (HHP):** 400 MPa for 10 and 15 min, 500 MPa for 10 and 15 min and 600 MPa for 10 min **Indirect plasma treatment:** microwave plasma with gas air (flow of 20 SLM, T = 4000 K, and RH = 20%) for 2.5, 5, 10 and 15 min **Direct plasma treatment:** rf-driven atmospheric pressure plasma jet (27.12 MHz) with power consumption in the range of 20 W, gas composition Ar + 0.10% O_2, shaking at 300 rpm with a distance to the sample of 24 mm for 0, 1, 2.5, and 5 min and 5 min		**Drying (10 and 15 min)**	**Vacuum cooking (90°C to 10 and 15 min)**	**HHP (400 Mpa 10 and 15 min)**	**HHP (500 Mpa 10 and 15 min)**	**HHP (600 Mpa 10 min)**	**HHP (600 Mpa 15 min)**	**Indirect plasma**	**Direct plasma**	
				Total microbial count	Inactivation of two log cycle	Inactivation of three log cycle	Inactivation of one log cycle	Inactivation of two log cycle	Inactivation of three log cycle	Inactivation of one log cycle	No effective	No effective	Rumpold et al. (2014)

					<u>With fecal contact</u>					**<u>Without fecal contact</u>**					
					Control (0 h)	**Control (24 h)**	**Starvation (24 h)**	**Control (48 h)**	**Starvation (48 h)**	**Control (0 h)**	**Control (24 h)**	**Starvation (24 h)**	**Control (48 h)**	**Starvation (48 h)**	
7	Fresh and starved	Incubator (30 °C) ↓ Unstarved mealworm - control (0, 24 and 48 h) ↓ Starved (24 h and 48 h)	Starved larvae: with the fecal contact and without fecal contact (sieved out from a batch during starvation to avoid contact with their fecal)	Total viable aerobic count	7.9 ± 0.2	8.0 ± 0.3	7.9 ± 0.4	8.0 ± 0.3	7.9 ± 0.5	7.9 ± 0.2	8.0 ± 0.3	7.8 ± 0.4	8.0 ± 0.2	7.8 ± 0.3	Wynants et al. (2017)
				Enterobacteriaceae	7 ± 0.3	7.0 ± 0.3	7.1 ± 0.3	6.8 ± 0.7	7.0 ± 0.5	6.9 ± 0.3	6.9 ± 0.4	7.1 ± 0.5	7.0 ± 0.3	7.1 ± 0.3	
				Aerobic bacterial endospores	1.5 ± 0.6	1.5 ± 0.3	1.4 ± 0.7	1.2 ± 0.3	1.2 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	1.7 ± 0.3	2.0 ± 0.3	1.6 ± 0.4	
				Psychrotrophic aerobic count	6.7 ± 0.4	6.4 ± 0.6	6.5 ± 0.4	6.5 ± 0.5	6.1 ± 0.3	7.0 ± 0.3	6.8 ± 0.2	6.8 ± 0.2	6.9 ± 0.3	7.1 ± 0.3	
				Yeasts and molds	5.6 ± 0.8	5.4 ± 0.6	5.3 ± 1.1	5.6 ± 0.8	5.8 ± 0.5	6.2 ± 0.4	6.3 ± 0.2	5.7 ± 0.4	6.2 ± 0.3	6.4 ± 1.0	

2.5 DRYING

Probably the oldest, the most important and the most widely applied method for food preservation drying (or dehydration), is a unitary operation that consists of the removal of moisture from a product, resulting from the simultaneous heat and mass transfer process due to the application of heat. It is a versatile and widespread technology in the food industry (LEE; JANGAM; MUJUMDAR, 2013; ONWUDE et al., 2016; SILVA et al., 2014).

Removing water from solid foods is a way of preserving, inhibiting the growth of microorganisms, and preventing a large part of the biochemical reactions that occur in the presence of moisture. It also reduces costs related to the transportation, packaging, and storage of high water content foods (PARK; BIN; PEDRO REIS BROD, 2003).

Food drying is a complex phenomenon involving mass and energy transport. Heat transferred from the drying medium to the wet solid can be by convection, conduction, or radiation and, in some cases, a combination of the three heat transfer mechanisms. Mass transfer depends on the transport of moisture inside the solid and on the transfer of water vapor from the product surface to the outside (RATTI, 2008).

Water on the surface of the product is removed and replaced by inner water by moisture transfer, a term used to describe a variety of processes such as diffusion, convection, capillary flow, physical deformation of food (shrinkage), among others. Vapor pressure increases with temperature as a result of continuous heat supply. Thus, heat transfer to the material being dried maintains the driving force for drying, that is, the difference in vapor pressure between the evaporating surface and the drying atmosphere (TROLLER, JOHN A.; CHRISTIAN, 1978).

Drying depends on process variables (temperature, relative humidity and drying air velocity), heat transfer mechanisms, and mass transport phenomena. The characteristics of the food matrix greatly influence the drying kinetics, due to its influence on the transport properties of the materials, being an essential part of the real mathematical model of any dehydration operation, which seeks an adequate estimate of the drying time involved (KROKIDA; PHILIPPOPOULOS, 2006).

Typical air-drying rate curve presents three periods of drying (GEANKOPLIS, 1998):
Adaptation period: the initial temperature of the product is lower than the temperature of the drying air, which rises rapidly to the wet-bulb temperature of the drying air. So the initial period is characterized by adapting the temperature of the product with the drying environment.

Constant drying rate period: the moisture removal occurs at a permanent rate because the product's internal moisture is transported to the surface at the same rate as evaporation occurs. The quantification of drying in this period is made by evaluating the energy received by the material being dried. This energy is equal to the energy required for vaporization of the water removed during drying. The moisture content at which the transition from the constant rate drying period to the falling rate period occurs is called "Critical Moisture Content" it is a unique feature of each process for a given product. In other words, the critical moisture content is not a universal value for a given product, as process conditions largely influence its value (COHEN; YANG, 1995; PARK; BIN; PEDRO REIS BROD, 2003).

Falling drying rate period: where the drying rate decreases as the moisture content decrease. This behavior is due to the predominance of internal resistance. It can occur at several different rates, depending on the moisture content of the product and the changes in its structure. During this drying period, the temperature of the product increases, reaching the temperature of the drying air. The minimum moisture that can be achieved is the equilibrium moisture of the material with the drying air.

Several drying techniques are applicable to dehydrate food, such as air-drying (AD) vacuum-drying (VD), freeze-drying (FD), and multi-flash drying (MFD).

2.5.1 Freeze-drying

The freeze-drying process has been considered one of the best for dehydrating high value-added thermolabile products (OIKONOMOPOULOU; KROKIDA; KARATHANOS, 2011). The technique is based on sublimation dehydration of the frozen product, which favors the maintenance of the product structure, resulting in foods with a highly porous structure, easily rehydrated and with retention of flavor and color. However, equipment costs, long drying times and high energy consumption make freeze-drying an expensive process (OIKONOMOPOULOU; KROKIDA; KARATHANOS, 2011; RATTI, 2008; VARNALIS; BRENNAN; MACDOUGALL, 2001)

2.5.2 Vacuum-drying

For foods that can suffer damages or even losses of vitamins and antioxidants if exposed to high temperatures, the vacuum-drying is one of the techniques widely used. It increases the rate of evaporation due to the decrease in the saturation temperature of the water. The maintenance of low temperatures is essential for thermo-sensitive products, in addition to establishing a drying environment with low concentrations of oxygen, contributing to reduce the sensorial and nutritional losses of the dehydrated products (ALIBAS, 2007).

The benefits of vacuum-drying include the use of lower process temperatures and higher drying rates, which result in better quality products, nutrient retention, and aromas when compared to hot air drying (ŠUMIĆ et al., 2013).

In vacuum-drying, removal of moisture from food takes place under low pressure. A thin layer of food is placed on a heated plate which supplies latent heat required for evaporation of water from the food. Hot water is normally used as the heat transfer medium (PAP, 1995).

Since the removal of moisture takes place in the absence of oxygen, oxidative degradation, e.g. browning, is low in the final product. As the system temperature is kept below 75 ˚C, the materials that are sensitive to oxygen and heat, like fruits and vegetables, can be dried (PAP, 1995).

Vacuum expands air and water vapor present in the food and creates a frothy or puffed structure. This expanded structure provides a large area to volume ratio for good heat and mass transfer, consequently high drying rate (JAYA; DAS, 2003).

2.5.3 Multi-flash drying

The process is based on successive heating-vacuum-pulse cycles of the samples, suitable for the production of dried-and-crisp food with low moisture content and water activity. Drying by multi-flash drying (MFD) is disclosed in patent document PI1107173-7 filed by Laurindo, Porciuncula, and Zotarelli (2011) and Zotarelli et al. (2012). In this method, the heating step can be performed by hot air (convective multi-flash drying, CMFD) (ZOTARELLI et al., 2012), by contact with heated plates (conductive multi-flash drying, KMFD) (PORCIUNCULA et al., 2016; LINK et al., 2018) or by microwaves (microwave multi-flash drying, MWMFD)

(MONTEIRO et al., 2016). This procedure results in dried-and-crispy products in shorter processing times.

If moist foods are subjected to pressure reduction, some of the food's internal water evaporates, cooling the food to a temperature close to the water saturation temperature at the applied pressure (WANG; SUN, 2001). Whenever a portion of the liquid evaporates, an amount of heat equal to the latent heat of evaporation must be absorbed by the evaporated part, resulting in a reduction in the temperature of the product (WANG; SUN, 2001). The amount of steam generated is dependent on the temperature difference between the two steps (before and after vacuum application). It is this evaporation that causes the formation of the porous structure of the food. The maintenance of this structure depends on its hardening, which is dependent on the temperature and water content of the product (LOUKA; ALLAF, 2002).

The amount of product evaporated water (mass loss) during the application of a vacuum pulse (vacuum cooling) is given by Equation 1 (ZOTARELLI et al., 2012)

$$\Delta m_w = \frac{c_p . m_p . \Delta T}{\Delta H_v} \quad (1)$$

in which Δm_w is the mass loss (kg); c_p is the average specific heat of the product in the working temperature range (J kg^{-1} $°C^{-1}$); m_p is the initial mass of the product (kg); ΔT is the material temperature reduction during vacuum application and ΔH_v is the water vaporization specific enthalpy at the final working pressure (kJ kg^{-1}).

Several drying techniques have been reported in the literature to the dehydration of YM larvae. Table 7 shows different drying processes with different experimental conditions and the significant results obtained. Up to now, the multi-flash drying technique has not been investigated in the drying of YM. Furthermore, MFD present low capital and operational costs and can be an alternative to conventional technologies in many situations (Porciuncula, Segura, & Laurindo, 2016; Zotarelli, Porciuncula, & Laurindo, 2012; Monteiro, Carciofi, & Laurindo, 2016).

Drying generally leads to a decrease in water activity and moisture content, which is one of the most important parameters to stabilize the final product.

Table 7 - Different drying process of *Tenebrio molitor*.

Drying and experimental conditions	Significant results	References
FD - Frozen (- 35 °C - 12 h), FD condenser at - 15°C - 30 h **AD -** 60 °C - 6 h **RO -** 180 °C – 11 min **PF -** 100 °C – 12 min **DF -** 250 °C – 2 min **ST -** 100 °C – 30 min **BO -** 100 °C – 10 min **MW -** 2.5 min	FD was the process that most preserved the content of proteins, minerals, and vitamins. *T. molitor* by DF and MW present the lowest content of total minerals and vitamins (B_1 and B_3), respectively.	Baek et al. (2019)
FD - 100 g YM, FD condenser at - 50 °C - 24 h **VD -** 300 g YM; 60 °C - 24 h **RRO -** 500 g YM; 120 ° C - 1h	Color impressions and volatile compound profiles were dependent on processing procedure. High-temperature in RRO process caused pronounced darkening with rather low content of volatiles, leading to the progress of Maillard reaction. VD or FD resulted in enrichment of volatile Maillard reaction and lipid oxidation intermediates. The total zinc contents for the differently dried mealworm samples were comparable to fresh larvae. The dried larvae had low zinc bioaccessibility (RRO - 20% and FD and MW - 40%).	Kröncke et al. (2019)
FDB - 5 kg YM; 130 °C - 110 min **MW -** 150 g; 850 W - 10 min **FD -** 200 g YM; Frozen (- 21 °C); FD Condenser at - 50 °C - 24 h **VD -** 350 g YM; 60 °C - 24 h **RRO -** 800 g YM; 120 °C - 1 h	Small differences were found between dried and fresh larvae in nutritional value. Protein solubility was highest at FD and VD. FD larvae significantly showed the highest oxidative status. Drying with a VD and MW can be an alternative to conventional freeze-drying for mealworms.	Kröncke, Böschen, Woyzichovski, Demtröder, & Benning (2018)
MW - 2 kW - 30 s, then for 3 kW - 5 min, 1 kW - 10 min and 0.5 kW -2 min **FD -** 2 min at 0.5 kW, YM were frozen (- 21 °C h) and FD for 52 h **MWVD -** P = 1 MPa, 1.5 kW - 6 min, then for 1 kW -10 min, 0.8 kW 4 min and 0.6 kW - 1min.	FD and MW generated minor changes in protein, fat, and ash content of the larvae. There was no statistic difference between the FD and MW drying for protein, fat, and ash content. The fatty acid composition and fat oxidation status were different for the larvae FD and MW. MW reduced the vitamin B_{12} content of YM compared to fresh, FD, and blanched plus FD samples. The browning indexes of MW larvae were stable over 4 months in contrast to that of FD samples. MW has some advantages over FD and is to be preferred in applications where color is essential. The YM blanched and dried by MWVD was more susceptible to oxidation than MW blanched samples. MWVD did not bring benefit since the fatty acid composition, vitamin B_{12} content, and the color was not almost influenced.	Lenaerts, Borght, Callens, & Campenhout (2018)
AD - 50, 60 and 70°C **FD -** Frozen (- 50 °C), FD - 55°C, P=0.2 mbar for 96 h	Guggenheim-Anderson-de Boer model well fitted experimental data, showing isotherms of type II and estimating a monolayer value of 0.05 g H_2O/g dry matter, appropriated for food stability and powdering operations. Blanching changed the adsorption isotherms, augmented the moisture of fresh larvae with no effect on their proximate composition. FD preserved better the color of fresh larvae, but when rehydrated, the FD samples produced higher color degradation.	Azzollini et al., (2016)

Freeze drying (FD); Air-drying (AD); Oven-broil (OV); Roast (RO); Pan fry (PF); Deep fry (DF); Steam (ST); Boil (BO); Microwave (MW); Vacuum drying (VD); Rotating rack oven (RRO); Fluidized bed drying (FBD); MW dried with vacuum (MWVD).

2.6 FINAL PRODUCT QUALITY

2.6.1 Water activity and moisture content

Water is one of the main constituents of food, which affects safety, stability, quality, and physical properties. The influence of water on food properties results from the interaction between water molecules and the other components of the food. The extension and intensity of interactions depend on the chemical composition and is determined by the state of the water in the food (LEWICKI; JAKUBCZYK, 2004).

Generally, the main purpose of food dehydration is to extend their life and get products with special features. This is achieved by reducing the water activity (a_w) of food at a value that will inhibit the growth and development of pathogenic and spoilage microorganisms, significantly reducing enzymatic activity and the rate at which undesirable chemical reactions occur. Food water activity is determined by (Equation 2), as the relationship between the vapor pressure of water contained in food by the saturated water vapor pressure at the same temperature (FELLOWS, 2009):

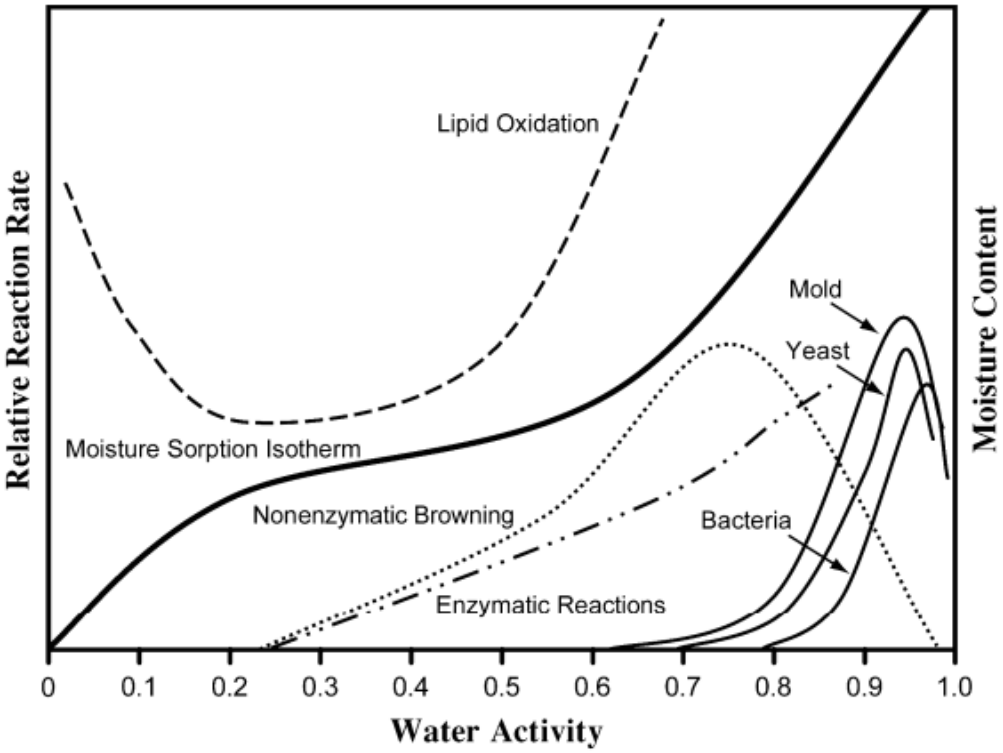
$$a_w = \frac{P}{P_0} \tag{2}$$

in which P is the vapor pressure of the water contained in food and P_0 is the pressure of pure water vapor at the same temperature.

Foods are classified, according to water activity, in three groups: low moisture foods ($a_w \leq 0.60$), intermediate moisture foods ($a_w$between 0.60 and 0.90) and high moisture foods ($a_w \geq 0.90$).

Drying has the main objective to decrease the water activity (a_w) of various perishable foods, allowing their further storage and controlling their shelf-life at ambient temperature by inhibiting the growth of micro-organisms, slowing down the rates of chemical reactions, and by reducing or suppressing enzymatic deterioration. Labuza et al. (1970) summarized that relationship in a diagram similar to that of Figure 5.

Figure 5 - Stability map of food as a function of water activity.



Source: Labuza et al. (1970)

In foods with high water content ($a_w \geq 0.90$), diluted solutions can be formed with the food components, which will be substrates for growth microbial. In this range of water activity, chemical and enzymatic reactions can have their velocity reduced due to the low concentration of reagents. Microorganisms easily contaminate foods in this condition. In addition to microbial growth, foods are also susceptible to oxidation, non-enzymatic browning (Maillard reactions) and enzymatic reactions (RIBEIRO, ELIANA PAULA; SERAVALLI, 2003).

Chemical reactions including lipid oxidation and non-enzymatic browning, are affected by water content. The rate of non-enzymatic browning or oxidation depends on system components, system viscosity, reagent dilution, and reagent mobility. For enzymatic browning, the maximum reaction rate occurs in an a_w range between 0.6 and 0.8 (KIM; SALTMARCH; LABUZA, 1981)

Drying decreases water activity and reduces, in turn, the microbial growth and the rate of chemical and enzymatic reactions (LABUZA, 1980; SABAREZ, 2015), lipid oxidation (a_w ranges between 0.20 and 0.35). Furthermore, it also increases nutrients contents as protein, fat, fiber, and minerals by reducing moisture content (SIEMIANOWSKA et al., 2013; TAOUKIS; RICHARDSON, 2007; ZHENG et al., 2013).

Moisture and water activity are important factors in ensuring the stability of the food (even though water activity is more important for food stability than the moisture content) due to microbiological and chemical transformations. For this reason, it is important to know the relationship between these factors (BARBOSA-CANOVAS et al., 2007).

At the same time, drying can also enable reactions inside the product that can lead to color change.

2.6.2 Product color

For having an important role in the appearance, the processing, and the acceptability of food, the color of food is one of the most significant quality factors. It determines the visual appearance of food, which can highly impact the choice of the consumer. It is the visual acknowledgment and assessment of the surface and subsurface characteristic of the food material (TIJSKENS; SCHIJVENS; BIEKMAN, 2001).

During drying, reactions that take place inside the food material (pigment degradation, especially carotenoids and chlorophyll, and browning reactions such as Maillard condensation of hexoses and amino components and oxidation of ascorbic acid, etc.) lead to the color change (BARREIRO, J.A.; MILANO, M.; SANDOVAL, 1997; LOZANO; IBARZ, 1997). The final values of color parameters can, therefore, indicate quality and be an auxiliary to evaluate the deterioration due to thermal processing (SHIN; BHOWMIK, 1995).

The color change in food materials can be determined in an indirect way. Colour parameters (L= from whiteness to darkness; a= from redness to greenness; and b = from yellowness to blueness) are used to describe visual color deterioration and to provide useful information for quality control in food products. The total color change (ΔE) and chroma, which indicates color saturation and is proportional to its intensity, are also determined afterward. (GARZA et al., 1999; TIJSKENS; SCHIJVENS; BEEKMAN, 2001).

The study of the color change behavior of YM during drying has recently been a subject of interest for many researchers. This is the case of Lenaerts et al. (2018), Kröncke et al. (2019) and Azzollini et al. (2016) who investigated the quality of the samples and to evaluate the intensity of the thermal treatment they are submitted.

3 MATERIAL AND METHODS

The experiments were performed at the Laboratory of Physical Properties of Food (PROFI) and at the Laboratory of Bioprocesses of Food Science and Technology Department, Federal University of Santa Catarina (UFSC).

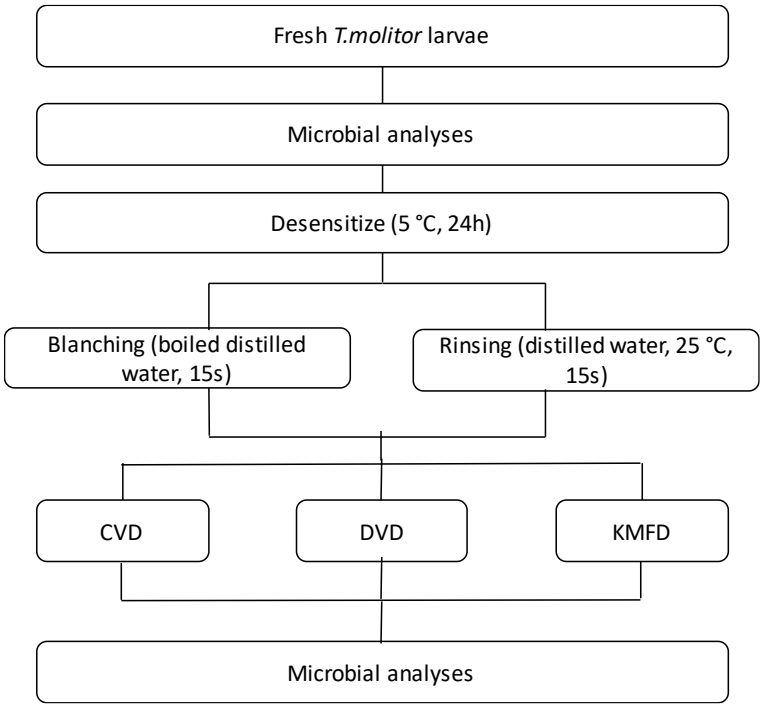
3.1 INSECT SAMPLES AND REARING

Samples of YM larvae were obtained from different companies in São José – Brazil, MG-Tenébrios and Tudo para o seu pet. The larvae purchased were submitted to rearing in plastic boxes (28 cm x 30 cm x 43 cm) at room temperature and relative humidity of approximately 25 °C and 60%, respectively. They were fed once a month with wheat bran and twice a week with fresh fruits and vegetables as a source of water (apples, bananas or potatoes). Another recipient was used to separate the adults from the larvae, which were reared for a period varying from 11 to 13 weeks (mature larvae). Furtherwards, a small number of larvae were reared apart to obtain new adults and replace the dead ones. Four batches were used in total in this study; they were sometimes mixed with our reared ones.

3.2 EXPERIMENTAL PROCEDURE

Figure 6 shows the schematic diagram of the experimental procedure. The larvae were first placed in a refrigerator (5 ˚C for 24 h) in order to desensitize them. Then 15 g of samples were rinsed (1 L of distilled water, 25 °C, 15 s) or blanched (1 L of boiled distilled water, 15 s). Finally, they were placed for 1 min on a filter paper to remove the residual water. The blanched or rinsed (non-blanched) YM were placed in an aluminum cylindrical container (63 mm x 53 mm), which was placed in the drying chamber, receiving conductive heating at the base. The YM were dried by three different techniques: the conductive vacuum drying (CVD), the conductive multi-flash drying I (KMFD), and the conductive multi-flash drying II (DVD). All drying experiments were performed in triplicate.

Figure 6 - Schematic diagram of the experimental procedure

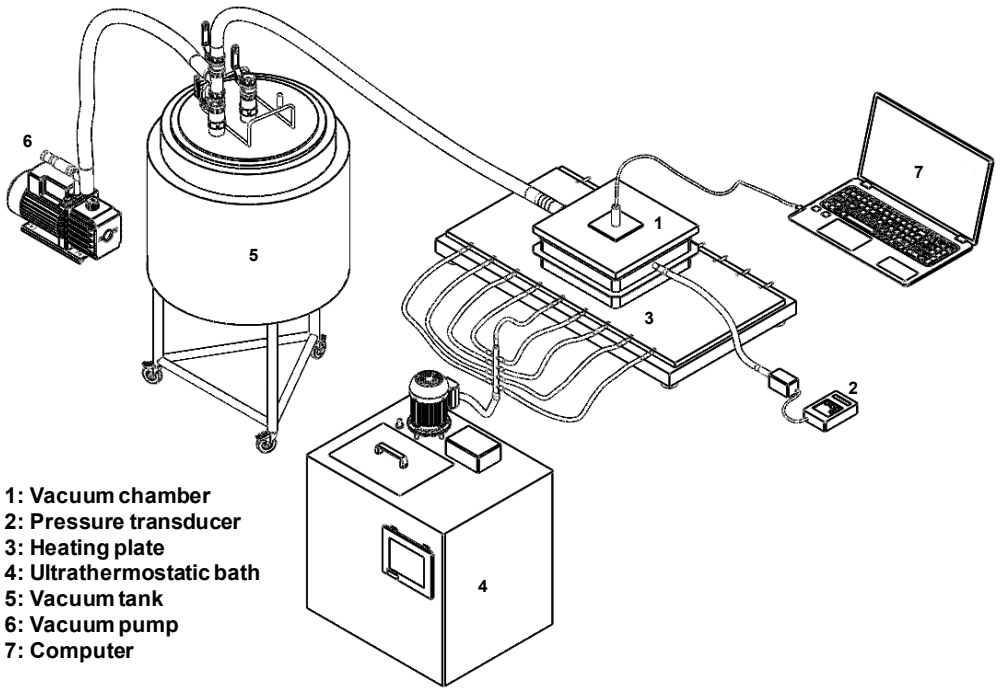


For all drying processes, the same experimental drying device was used (Figure 7). The equipment consisted of a stainless heating metal plate (80 cm x 40 cm), heated by hot water (96 ± 2 °C) coming from an ultra-thermostatic bath (Callmex, model - 6214M2, Brazil) that provides the same temperature condition for the entire plate. The stainless steel vacuum chamber (300 mm x 195 mm x 45 mm) with a steel cover (19 mm thick) was coupled to a vacuum pump with a nominal flow rate of 10.2 $m^3 h^{-1}$ (DVP, Vacuum Technology, Model - RC.8D, Italy). In this study, a vacuum reservoir (53.5 L) was connected to the vacuum pump to assist in the rapid decompression of the system. Pressure control was performed with the aid of a pressure transducer (ILMVAC, model - 600071 Grobvakuummeter, Germany). Before each drying experiment, the equipment was cleaned with neutral soap and alcohol 70% to avoid additional contamination.

Samples temperature during the dryings was measured by an infrared thermometer (Contemp, Model - CT-SF15-C3, Brazil) inserted at the center of the chamber cover and connected to the computer.

The experimental apparatus is presented in Figure 7. Full details of the equipment can be found in Maciel (2019).

Figure 7 - Experimental drying device.



Source: Maciel, 2019

3.2.1 Conductive Vacuum Drying (CVD)

For CVD, the samples were placed in the vacuum chamber and the pressure was immediately reduced to 4 kPa (sudden decompression) and maintained so during the entire process.

3.2.2 Conductive Multi-flash Drying I (KMFD)

For KMFD, the samples were placed in the vacuum chamber and heated up to 60 °C at atmospheric pressure, then the pressure was immediately reduced to 4 kPa (sudden decompression) and maintained so for 2 minutes. After the 2 minutes, the atmospheric pressure was re-established and a new cycle was started. After the fifth cycle, the pressure was reduced to 4 kPa and maintained so until the end of the drying.

3.2.3 Conductive multi-flash drying II (DVD)

For DVD, the samples were placed in the vacuum chamber and the pressure was immediately reduced to 4 kPa (sudden decompression). At regular intervals of 10 min, the pressure was re-established to atmospheric pressure then immediately reduced again, repeatedly until the end of the drying. At the same regular intervals, the set aluminum cylindrical container plus samples was weighed in an analytical balance, and the data were used to plot the drying curves.

The process parameters of all the drying techniques were performed in several trials to determine the drying times or final temperatures that were necessary to reach a water activity ranging between 0.2 and 0.3. Drying times occurred to be more precise than final temperatures. Further experiments were then performed based on the drying times previously established.

The drying curves were obtained by weighing the set aluminum cylindrical container plus samples before placing the it in the vacuum chamber and after every vacuum pulse (KMFD) or after every 10 minutes (DVD) or only at the end of the drying (CVD).

The empirical model of Midilli et al. (2002) was adjusted to the experimental data of the drying curves, represented by the variation of moisture (dry basis) over time. The model is represented by Equation 3:

$$y = ae^{-kx^n} + bx \tag{3}$$

in which, y are the measures of moisture, x is the drying time and a, k, n, and b are the coefficients and empirical constants.

For each drying experiment, the moisture, a_w and color of the samples (fresh, blanched, and dried) were measured in triplicate. The moisture content was determined by the gravimetric method, placing the samples in a drying oven (SP Labor, Model - SP-100/150, Brazil) at 105 °C until constant weight (approximately 24h) (AOAC, 2002). With their final weight, the moisture content on a dry basis was determined using the following equation:

$$X_{db}(gg-1) = \frac{Initial\ weight - Final\ weight}{Final\ weight} \tag{4}$$

a_w was determined by crushing the samples and placing them in a digital hygrometer at 25 °C (Aqualab Model-Series 3 TE, Decagon Devices, Inc., Pullman, USA). The color parameters of the samples were determined using a computer vision system (CVS), according to the methodology described by Cárdenas-Pérez et al. (2017) with minor adaptations. Images

were captured using a camera (Nikon D5500, Nikon Corporation, Japan) with a resolution of 4496 × 3000 pixels and a white background camera-equipped with white light (D65 Lighting Standard). Digital images were treated by the software ImageJ v. 1.6.0 (National Institutes of Health, USA). The color space converter plug-in was used to convert colors from RGB system to CIELab scale, L* values (brightness) vary from white (100) to black (0), a* is defined as a transition from green (-a*) to red (+ a*) and b* represents the transition from blue (-b*) to yellow (+ b*). Total color variations were evaluated by the parameter ΔE*, calculated according to Equation 5:

$$\Delta E^* = \sqrt{(L^*-L_0)^2 + (a^*-a_0)^2 + (b^*-b_0)^2} \quad (5)$$

3.3 MICROBIAL ANALYSES

Microbial plate counts were determined according to the ISO standards for microbial analyses of food as compiled by Stoops et al. (2016) and Vandeweyer, 2017.

Five grams of fresh or dried YM larvae were transferred aseptically into a sterile stomacher bag and 45 ml of sterile physiological salt solution (0.85% (w/v) NaCl), Merck KGaA, Germany) was added. The mixture was homogenized for 60 s in a stomacher (Interscience, 400 W, P, n° 97034019, France). With the use of pipettes and micropipette tips, a ten-fold serial dilution series was plated on correspondent mediums using the spread drop plate technique (spreading the liquid over the surface of the medium plate with a sterile spreader stick, until the liquid was completely absorbed into the medium).

Total viable counts (TVC) were determined on Plate Count Agar (PCA, Biokar Diagnostic) incubated at 30 °C for 3 days. *Enterobacteriaceae,* on Violet Red Bile Glucose medium (VRBG, Biokar Diagnostic) incubated at 37 °C for 24 h. LAB, on de Man Rogosa Sharpe medium (MRS, Biokar Diagnostic) incubated at 30 °C for 3 days in the absence of oxygen. Yeasts and molds on Oxytetracycline Glucose Agar (OGA, Biokar Diagnostic) supplemented with oxytetracycline (50 mg/550 ml OGA, Biokar Diagnostic) incubated at 25 °C for 5 days. Bacterial endospores were determined by giving the 10^{-1} dilution a heat shock treatment (10 min at 80 °C), followed by serial dilution, plating onto PCA and incubation at 37 °C for 24 h.

As for the pathogenic, *E. coli* counts were determined after aerobic incubation on TBX (Tryptone Bile X-Glucuronide) for 24 h at 44 °C, *B. cereus* counts were determined after aerobic incubation on MYP (Mannitol Yolk Polymyxin) agar (30 °C, 24 h and 48 h), and *Salmonella sp.* counts were determined by the method for *Salmonella sp.* detection in foods, according to the ISO 6579 standard.

All microbiological experiments were performed in duplicate with two repetitions for initial (fresh), and final counts (dried) and the average were expressed as log cfu/g.

3.4 CHALLENGE TEST WITH *E. COLI*

E. coli is widely disseminated in the environment through the feces of humans and other animals, coupled with its ability to survive out of the colon for months (KRUMPERMAN, 1983; WEARY et al., 1972). This bacteria can, then, potentially be present in environment YM are reared or feed they are fed (organic waste) and, consequently, contaminate them.

For the evaluation of the effectiveness of CVD processes against *E. coli*, surrogate strains of *E. coli* ATCC 8739 and ATCC 25922 were pooled and inoculated into the larvae feed before treatment. The challenge test was performed using wheat bran for YM larvae feed. The wheat bran was inoculated with the *E. coli* strains pool. Fresh live YM were then placed in the contaminated bran (YMB) to investigate the occurrence of *E. coli* contamination along with TVC and *Enterobacteriaceae* in 24 h and in 7 days.

The *E. coli* stock cultures were maintained on Brain Heart Infusion agar (BHI) (Acumedia, Lansing, Michigan, USA) slants at 4 °C. The cultures were subcultured twice from the stock in nutrient broth (NB) (Oxoid, Basingstoke, Hampshire, England) and cultivated overnight at 35 °C until reaching 9 log cfu/ml. Then the cultures were centrifuged (4.000 g for 10 min), and the supernatants were discarded. The cells' pellet was washed using physiological salt solution. The inoculum was plated onto Eosin Methylene Blue agar (EMB) (Acumedia) and incubated for 24 h at 35 °C to confirm whether it did contain 9 log cfu/ml of *E. coli* cells in the undiluted inoculum. After confirmation, the *E. coli* pool was diluted until the desired concentration for inoculation on the wheat bran (low inoculum = 3 log cfu/g and high inoculum = 7 log cfu/g).

The inoculation of the wheat bran was performed in a sterile stomacher bag by pouring 20 droplets of 50 µl with the desired log concentration in 300 g of bran in each replicate,

followed by stomacher homogenization for 3 min. The inoculated bran was placed into sterile aluminum trays (5L, 5×17×24 cm), added 50 g of live YM and placed in a culture chamber for 24 h or 7 days at 25 °C and 68% of relative humidity. The microbial counts of the wheat bran and YM were performed before the contamination, after 24 h and 7 days of exposure of YM to the contaminated wheat bran and after YMB were submitted to CVD, as described in section 3.3 for TVC, *Enterobacteriaceae* and *E. coli*. Replicate counts in cfu were converted to log values and expressed as averaged log numbers.

3.5 STATISTICAL ANALYSIS

The results of water activity, moisture content, color, and microbiology were analyzed statistically with Statistica 7.0 program (StatSoft, Tulsa, USA), using analysis of variance (ANOVA) and Tukey test at 95% confidence level ($p \leq 0.05$).

4 RESULTS AND DISCUSSION

4.1 DRYING CURVES

Table 9 shows the average moisture content of fresh, blanched, and dried YM, the initial and final water activity, and total drying time (t_f) for different drying processes. Fresh samples of YM used in this study presented initial moisture content (X_{dbo}) ranging from 1.5316 ± 0.0367 to 1.8099 ± 0.0071 g g^{-1} (dry basis, db) and water activity (a_{w0}) ranging from 0.980 ± 0.003 to 0.988 ± 0.000. The blanched samples presented X_{dbo} ranging from 1.4562 ± 0.0540 to 1.9044 ± 0075 g g^{-1} (db) and a_{w0} ranging from 0.986 ± 0.009 to 0.990 ± 001.

The use of the same batch was not possible because of the scarcity of YM larvae during the period of this study. It was then necessary to buy larvae from different producers. Those larvae were consequently exposed to different diets and relative humidity, which explains the difference in their initial moisture content, as YM can obtain water from both the food ingested and the atmosphere (FRAENKEL & BLEWETT, 1944; NOWAK et al., 2014). The same can explain the fact that fresh YM from batch 3 and 4 presented the highest moisture contents. The X_{db} and a_w of rinsed and blanched YM are all in agreement with those of other studies published (MELIS et al., 2018; LENAERTS et al., 2018; VANDEWEYER et al., 2017; SIEMIANOWSKA et al., 2013; YI et al., 2013).

After drying processes, the final moisture content X_{dbf} and water activity a_{wf} were reduced to values equal or less than 0.0315 g g^{-1} and 0.287 g g^{-1}, respectively. Thus, these dried YM are very less or not exposed to from bacteria and yeast and mold growth, lipid oxidation, nonenzymatic browning, (BELITZ, H. D., GROSCH, W., & SCHIEBERLE, 2009).

Blanching is mostly applied as a pretreatment for fruits and vegetables to disable enzymes and to decrease the microbial load before further storing, processing or packing (FELLOWS, 2009; XU et al., 2012). In this study, a plus of blanching YM prior to drying was its influence on the reduction of drying times. It was noticeable that the blanched samples reached the targeted range of water activity in shorter times (around 30 min less) and lower temperatures than the rinsed ones. A hypothesis for this behavior is that blanching facilitated the removal of water from the YM cells during the drying.

Figure 8 depicts the drying curves of blanched and rinsed YM dried by DVD, KMFD and CVD processes. All the drying experiments were performed in triplicate and showed good reproducibility.

The temperature peaks close to 97 °C are related to the plate temperature when the set “aluminum cylindrical container plus samples” was removed for weighing to obtain the drying curves.

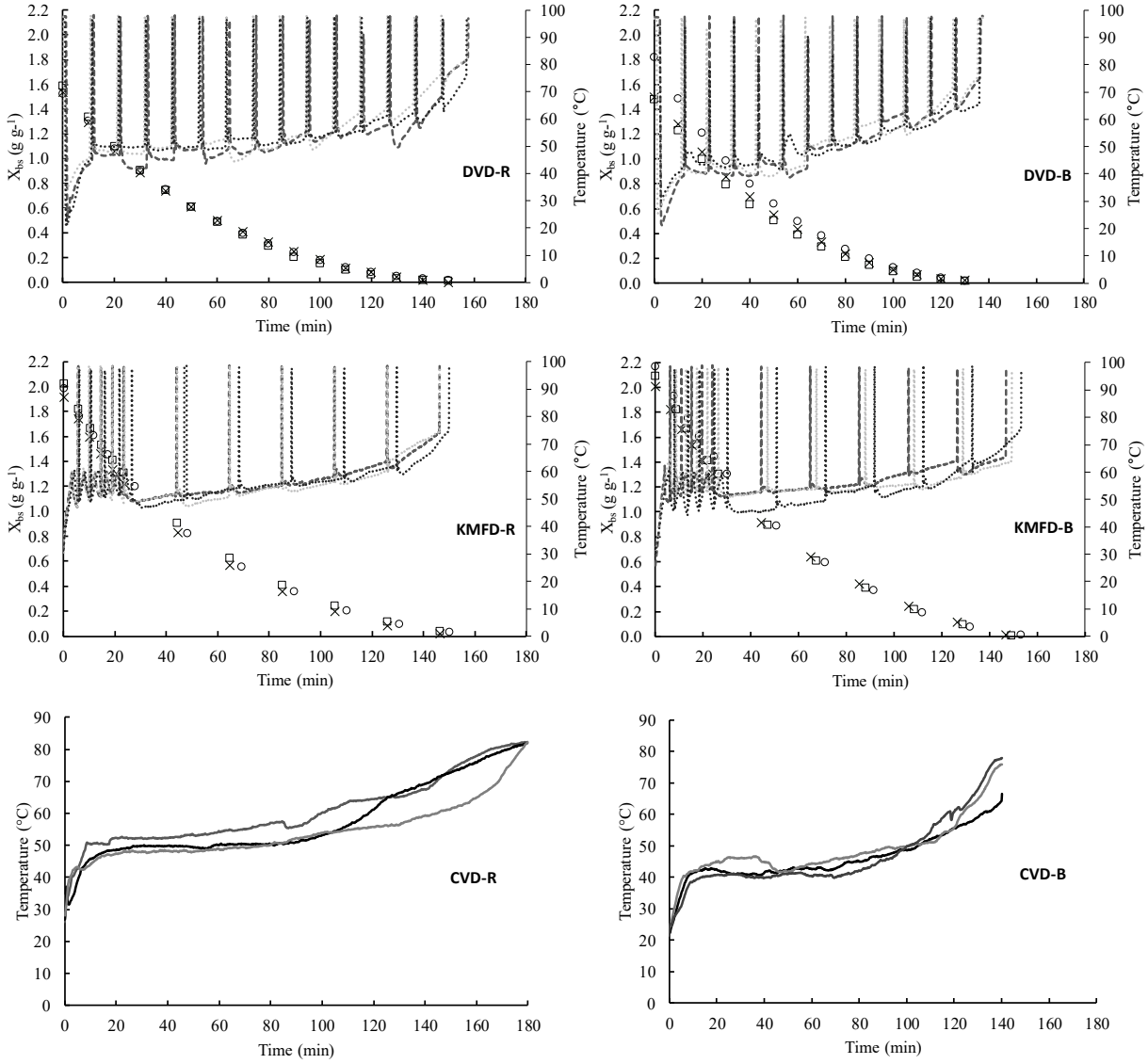
To reach the water activity between 0.3-0.2, it was necessary to apply at least 13 vacuum pulses for DVD drying, while for KMFD drying, it was necessary 5 heating-vacuum pulse cycles.

Table 8 - Initial and final moisture contents (db), water activities and the drying times for each drying technique.

Batches	Technique	X_{db0} (g g^{-1})	a_{w0}	X_{dbf} (g g^{-1})	a_{wf}	t_f (min)
1	CVD - B	1.5208 ± 0.0226^{a*}	0.986 ± 0.009^{a}	0.0172 ± 0.0730^{ab}	0.287 ± 0.022^{a}	140.00 ± 0.00
	CVD - R	1.5493 ± 0.0660^{a}	0.980 ± 0.003^{b}	0.0134 ± 0.0380^{ab}	0.207 ± 0.004^{b}	180 .00± 0.00
2	DVD - B	1.4562 ± 0.0540^{a}	0.986 ± 0.009^{a}	0.0222 ± 0.0510^{ab}	0.208 ± 0.007^{b}	130.00 ± 0.00
	DVD - R	1.5316 ± 0.0367^{a}	0.980 ± 0.003^{b}	0.0143 ± 0.0600^{ab}	0.208 ± 0.140^{b}	150.00 ± 0.00
3	KMFD - B	1.9044 ± 0.0075^{b}	0.990 ± 0.001^{c}	0.0111 ± 0.0095^{a}	0.265 ± 0.014 ^{a}	149.56 ± 2.56
4	KMFD - R	1.8099 ± 0.0071^{b}	0.988 ± 0.000^{ac}	0.0315 ± 0.0125^{b}	0.261 ± 0.025 ^{a}	147.52 ± 2.11

*$^{a, b}$ Means followed by different letters in the same column represent significant differences ($p \leq 0.05$) according to Tukey's test. B= Blanched; R= Rinsed.

Figure 8 - Temporal evolution of the moisture (dbs) of the YM rinsed and blanched during drying (DVD and KMFD); and time evolution of the sample temperature during drying DVD, KMFD, and CVD.



The utilization of vacuum drying guarantee not only a drying environment with low concentration of oxygen, but also the decrease of the saturation temperature of the water that leads to an increase in the rate of evaporation without reaching very high temperatures, which in turn contribute to reducing the sensorial and nutritional losses (ALIBAS, 2007). The exposure at high temperatures and at long processing times can not only degrade some heat-sensitive nutrients, but also favor browning reactions and tissue collapse that in turn lead to the darkening and shrinkage of the YM (PURSCHKE, BRÜGGEN, SCHEIBELBERGER &

JÄGER, 2017). The temperatures and processing times in this study were relatively lower than those used for other drying studies. Oven and fluidized bed drying, for example, has resulted in longer drying times (7 h- 24 h) (PURSCHKE, B., BRÜGGEN, H., SCHEIBELBERGER, R., & JÄGER, 2017). In the study of Kröncke et al. (2018), it required temperature of 130 °C to dry YM to an a_w of 0.56 with the fluidized bed drying process.

Figure 9 shows the experimental drying curves (dimensionless moisture content) of YM dried by DVD and KMFD, as well as the Midilli model adjusted to the experimental data of the drying curve. The Midilli model was adjusted to the experimental data, showing an adjusted determination coefficient (R^2) higher than 0.99 for all the cases. Figure 10 presents the experimental drying rate and the drying rate from the Midilli model. The curves were represented in triplicate and showed good reproducibility of the results.

For a better comparison of the drying processes, one curve from each experimental condition of dimensionless moisture content and drying rates was plotted (Figure11). The behaviors of DVD and KMFD were very similar. As time increased, falling rate periods were observed for all the processes. Similar behaviors were reported by Azzollini et al. (2016).

Figure 9 - Temporal evolution of experimental data of dimensionless moisture content and the adjustment of the Midilli model in triplicate. The dots represent de experimental data and the lines are the data predicted by the model.

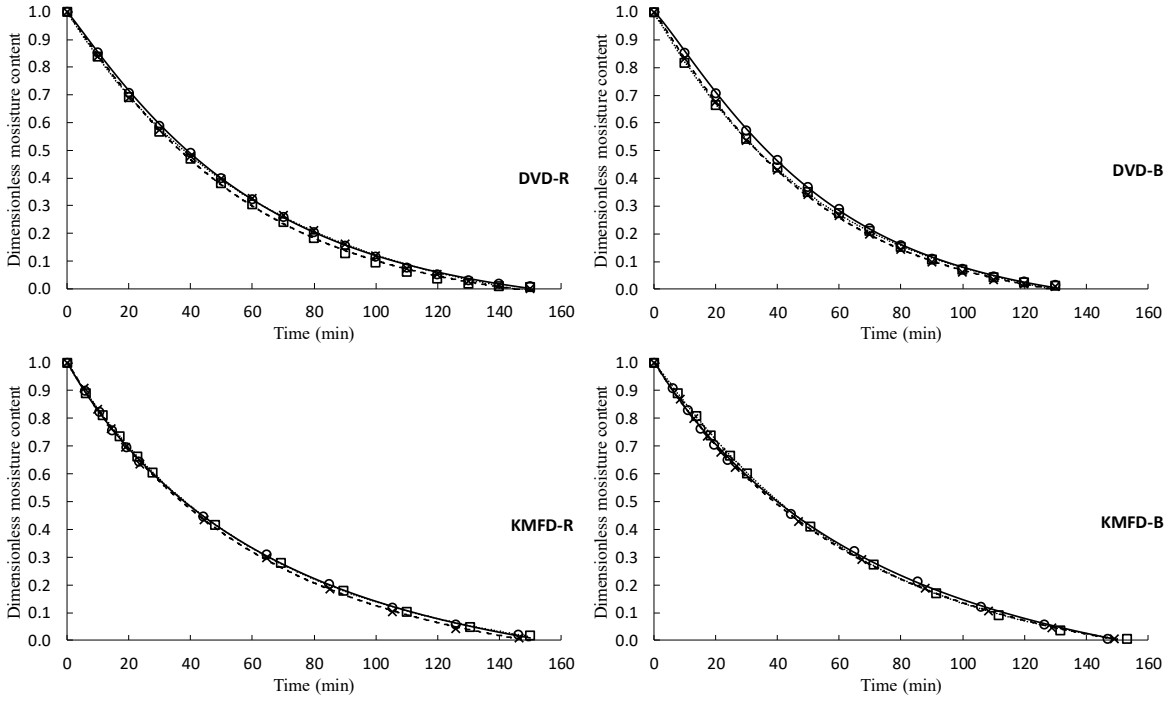


Figure 10 - Temporal evolution of experimental data of dimensionless drying rate and the drying rate from the Midilli model. The dots represent de experimental data, and the lines are the data predicted by the model.

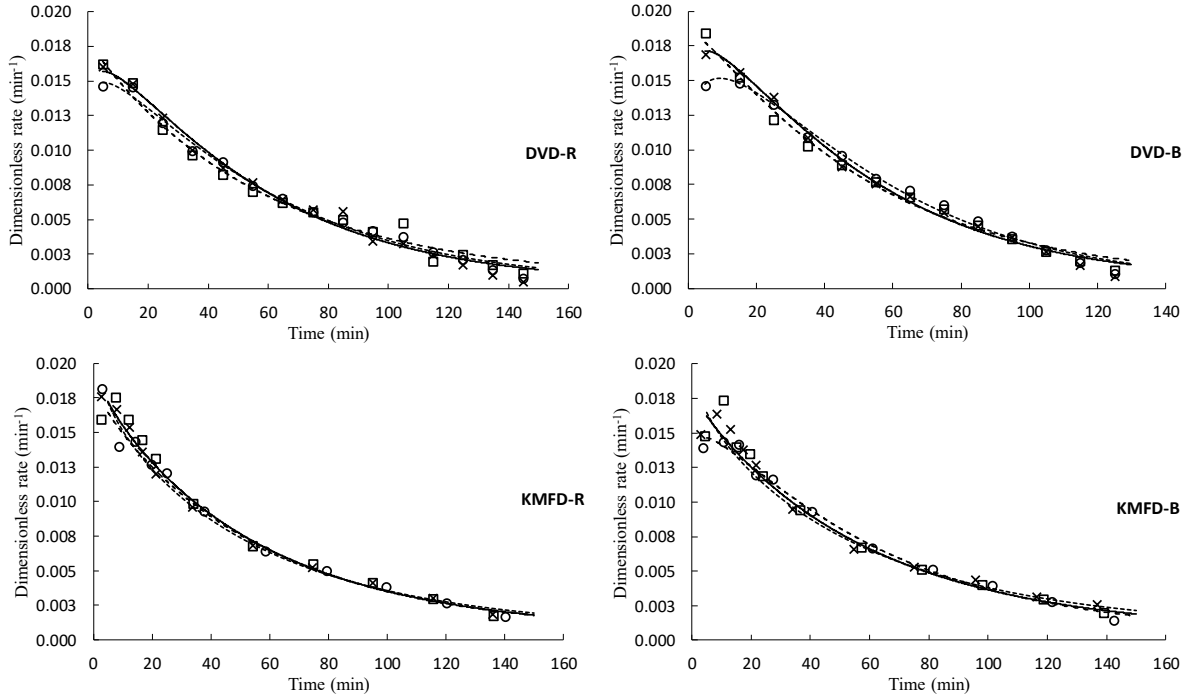
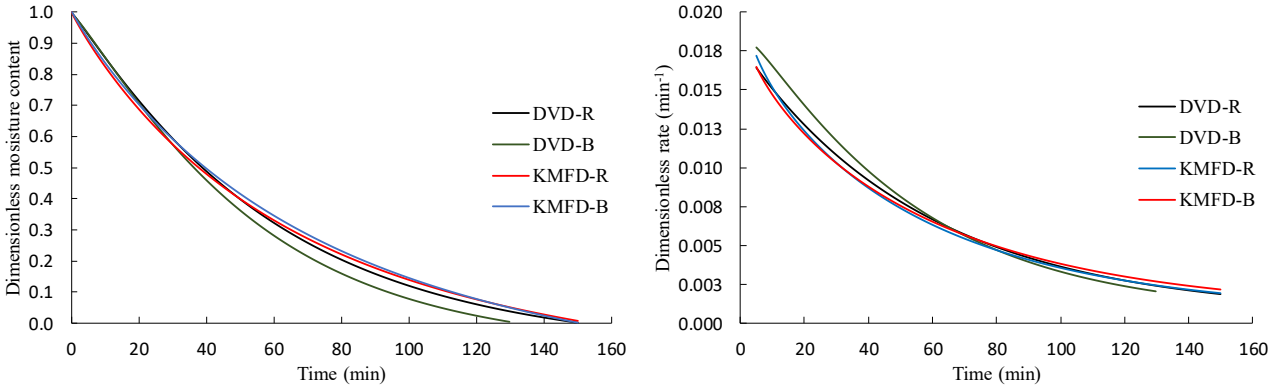


Figure 11 - Representative drying curves of blanched and rinsed YM dried by DVD and KMFD.



4.2 MICROBIAL ANALYSES

Microbial counts for fresh YM larvae before any treatments were very similar for the two batches investigated, as shown in Table 10. There were observed no statistical differences for the individual microbial counts between batches (except for the yeast and molds analyses). Except for LAB, yeast and molds, and aerobic bacterial endospores, the range of all the counts

(included pathogens) are in accordance with the results obtained by Caparros Megido et al. (2018), Klunder et al. (2012), Vandeweyer et al. (2017), Stoops et al. (2016) and Wynants et al. (2017). Differences between LAB, yeast and molds, and aerobic bacterial endospores counts of this study and others can be a result of YM being submitted to different feed supply and/or rearing practices, as microbiological parameters of YM depends on those variables (VANDEWEYER et al., 2017).

The effects of the combinations of rinsing plus drying and blanching plus drying on the microbiota of YM larvae were investigated, and the results are shown in Table 11. For the rinsed samples, it was noticed that the drying technique that reduced all the microbial loads the most was the DVD; that may be due to the highest number of vacuum pulses applied during the drying. KMFD reduced all the microbial loads considerably, but could not reduce LAB and yeast and molds loads to the same level as DVD, maybe because it had fewer vacuum pulses in the process.

The same can be observed with CVD, which did not present vacuum pulse in the drying, resulting in the lowest reduction loads of LAB and yeast and molds. Vacuum pulses demonstrated a significant and positive effect in disabling the microorganisms of YM during the drying process.

Table 9 - Microbial analyses of fresh YM.

Microorganisms	**Microbial counts (log cfu/g*)**	
	Batch 1	**Batch 2**
Total viable aerobic count	8.4 ± 0.6^{a}	7.5 ± 0.4^{a}
Psychrotrophic aerobic count	6.4 ± 0.8^{a}	7.3 ± 0.2^{a}
Lactic acid bacteria	6.1 ± 0.1^{a}	6.2 ± 0.2^{a}
Aerobic bacterial endospores	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Yeasts and molds	6.6 ± 0.1^{a}	5.9 ± 0.1^{b}
Bacillus cereus	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Enterobacteriaceae	6.6 ± 0.4^{a}	6.2 ± 0.2^{a}
Salmonella sp.	Absence in 25 g	Absence in 25 g
Escherichia coli	Absence in 25 g	Absence in 25 g

* Mean values ± standard deviation; [a, b] Means followed by different letters in the same line represent significant differences ($p \leq 0.05$).

For the blanched samples, all the three drying techniques were able to reduce the initial microbial loads to <2 log cfu/g. From those results, it can be interpreted that no matter which of these techniques is used to dry YM, if they are previously blanched then dried in the same conditions (time, temperature, pressure, and hygiene), their initial microbial loads will be reduced to at least <2 log cfu/g and there will be potentially no presence of *Salmonella spp*. and *E. coli* in 25 g of the samples. And by the same logic, blanching before drying is primordial to ensure a satisfying reduction of YM microbial loads.

Microbial spoilage of foods is caused by the growth and metabolic activity of various microorganisms. LAB, bacterial endospores, *Enterobacteriaceae*, yeasts and molds, and TVC are parameters used to measure the hygienic conditions or quality of food products. In this study, the combination of blanching and drying (CVD, DVD, or KMFD) was more efficient in reducing the initial YM microbial load than rinsing and drying. Only the blanching could already reduce the microbial load, and probably, exert stress on the microbial cell wall, disabling more easily the microorganisms during the drying. Rinsing, contrary to blanching, had no effect on the reduction of microbial load, as was proven not to lessen the microbial counts of the YM larvae in the study reported by Wynants et al. (2017). It can be considered that when processing YM larvae, one of the purposes of applying the blanching is to reduce their microbial load prior to further processing or storage (XU et al.,2012; VANDEWEYER et al., 2017).

According to Megido et al. (2017), the TVC limit for edible insects should be similar to the one for fresh minced meat, which means 6.7 log cfu/g as the limit. In this study, the initial TVC was much higher than the limit for fresh meat. However, it was reduced to a satisfying count for both rinsed and blanched YM larvae after drying.

Table 10 - Microbial analyses of YM after the pre-treatments (rinsed and blanched) and the different drying techniques.

Microbial counts (log cfu/g*)						
	CVD		DVD		KMFD	
Microorganisms	**Rinsed**	**Blanched**	**Rinsed**	**Blanched**	**Rinsed**	**Blanched**
Total viable aerobic count	2.0 ± 0.3^{a}	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Psychrotrophic aerobic count	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Lactic acid bacteria	4.6 ± 0.0^{c}	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	2.5 ± 0.4^{b}	$<2.0 \pm 0.0^{a}$
Aerobic bacterial endospores	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Yeasts and moulds	2.7 ± 0.2^{b}	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	2.3 ± 0.2^{b}	$<2.0 \pm 0.0^{a}$
Bacillus cereus	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Enterobacteriaceae	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$	$<2.0 \pm 0.0^{a}$
Salmonella sp.	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g
Escherichia coli	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25g	Absence in 25 g	Absence in 25 g

* Mean values ± standard deviation; [a, b] Means followed by different letters in the same line represent significant differences ($p \leq 0.05$).

4.3 CHALLENGE TEST WITH *E. COLI*

The inoculum level used in a microbiological challenge depends on the objective of the study. In our case, the 7 log cfu/g level was determined for validation of CVD process lethality to demonstrate the extent of reduction in the challenge. To our knowledge, no international D performance reduction standard for relevant hazardous microorganisms in edible insects was declared by regulatory agencies.

As shown in Table 12, before contaminating with *E.coli*, both the wheat bran and YM presented initial loads of TVC and *Enterobacteriaceae.* No *E. coli* contamination was detected. TVC load increased for both CL3 and CL7 on day 1, and decreased for CL7 on day 7, while *Enterobacteriaceae* load increased for both CL3 and CL7 on day 1 and day 7, respectively. However, the combination of blanching + CVD led to satisfying decontamination.

After contamination with *E. coli,* the contaminated wheat bran (CWB) presented *E. coli* load within the targeted contamination level (CL). The average counts for both batches after *E. coli* inoculum indicated 3,19 and 6,97 log cfu/g for CL3 (low inoculum) and CL7 (high inoculum), respectively. *E. coli* counts increased in 24 h in the CWB and YMB, probably due to their multiplication (exponential phase), then decreased in 7 days for CL3 and CL7.

Table 11 - Contamination with *E.coli* and the effect of CVD on the reduction load.

Samples	Contamination *E. coli* level (log cfu/g)	TVC (log cfu/g)			*Enterobacteriaceae* (log cfu/g)			*E. coli* (log cfu/g)		
		Initial	24h	7 days	Initial	24h	7 days	Initial	24h	7 days
CWB	3.00	5.30 ± 0.06	5.38 ± 0.03	5.72 ± 0.07	4.55 ± 0.11	5.76 ± 0.02	6.05 ± 0.07	0.0	5.57 ± 0.13	3.47 ± 0.01
	7.00		9.0	5.63 ± 0.54		6.27 ± 0.03	7.11 ± 0.00		6.15 ± 0.06	4.23 ± 0.03
YMB	3.00	8.00 ± 0.6	9.0	8.55 ± 0.04	6.04 ± 0.3	6.16 ± 0.13	7.00 ± 0.00	0.0	5.98 ± 0.04	4.00 ± 0.00
	7.00		9.2	8.72 ± 0.05		6.09 ± 0.08	7.15 ± 0.00		6.12 ± 0.01	4.90 ± 0.01
CVD-YMB	3.00		<2.00	<2.00		<2.00	<2.00		Absence in 25 g	Absence in 25 g
	7.00		<2.00	<2.00		<2.00	<2.00		Absence in 25 g	Absence in 25 g

CWB= *E. coli* contaminated wheat bran; YMB= larvae fed *E. coli* contaminated wheat bran; CVD-YMB= *E. coli* contaminated larvae dried by continue vacuum drying.

The decrease of *E. coli* in both the CWB and YMB on the 7th day at a lower level of contamination (CL3) than that in 24 h was probably due to the increase of *Enterobacteriaceae*. Some of the microorganisms of that family might have suppressed the growth and/or caused the decrease of *E. coli* by a possible competition. Another explanation to that can be the fact that YM are cold-blooded, which is not a favorable condition for the growth of *E. coli*; as this one grows, generally, in warm-blooded animals (BELL and KYRIAKIDES, 1998). By the mechanism of a general immune response, invertebrates are able to increase resistance to all pathogen types (RÂBERG et al. 1998). The dorsal homologue gene (TmDorX2) identified in YM, which could be considered as a positive regulator for the production of antimicrobial peptides against *E. coli* in gut, fat body, and hemocytes of young YM in response to bacterial and fungal infection, as described by Keshavarz et al. (2019), can then be another potential explanation for that decrease.

The combination of blanching and CVD presented efficacity in reducing TVC, *Enterobacteriaceae*, and *E. coli* loads of YM after *E. coli* challenge test, providing satisfying reduction results. CVD technique could deliver a 5 D lethality level. Blanched-dried YM counts for TVC and *Enterobacteriaceae* were all below 2.00 log cfu/g for all samples. Using CVD, as stipulated in this study, guarantee the safety of YM against *E. coli*. It not only reduces the load of *E. coli,* but it also prevents or minimizes the growth of that pathogen in YM by lowering water activity.

4.4 COLOR

Color is one of the most significant quality features in food, as it has an extremely important role in its visual appearance and its acceptability. The final values of the total color changes can indicate the level of deterioration caused by thermal processing (TIJSKENS et al., 2001; SHIN et al., 1995).

Figure 12 shows the pictures of fresh and dried YM, and Table 13 shows the average of color parameters (L*, a*, and b*) of fresh and dried YM. All the parameters of YM variated between the pre-treatments and drying techniques. The total color change (comparing with the fresh's color) was also presented (ΔE). A larger value of ΔE indicates a more shift from all initial colors.

Figure 12 - Pictures of fresh, rinsed-dried and blanched-dried YM.

Table 12 - Color parameters of fresh, rinsed-dried and blanched-dried YM.

	L*	a*	b*	ΔE
Fresh	$40.10 \pm 0.46^{a**}$	6.51 ± 0.28^{ab}	31.08 ± 1.03^{a}	
CVD-R	36.83 ± 0.33^{d}	7.21 ± 0.30^{b}	20.29 ± 0.38^{b}	11.29 ± 0.65^{a}
CVD-B	38.65 ± 0.53^{b}	5.66 ± 0.23^{a}	15.82 ± 0.77^{c}	15.34 ± 0.27^{bc}
DVD-R	35.10 ± 0.17^{e}	6.51 ± 0.41^{ab}	17.30 ± 0.77^{c}	14.65 ± 0.48^{abc}
DVD-B	36.27 ± 0.18^{d}	7.05 ± 0.95^{ab}	16.32 ± 1.37^{c}	15.26 ± 0.80^{bc}
KMFD-R	31.01 ± 0.26^{c}	7.31 ± 0.30^{b}	21.96 ± 1.15^{b}	12.89 ± 0.24^{ac}
KMFD-B	34.52 ± 0.27^{e}	7.21 ± 0.81^{b}	15.01 ± 0.58^{c}	17.02 ± 0.71^{b}

**[a-e]Means followed by different letters in the same column represent significant differences ($p \leq 0.05$) according to Tukey's test

These initial L* and a* values are close to that found by Lenaerts et al. (2018) for fresh YM, while the initial b* value was much higher. This difference can be explained by the hypothesis that YM larvae carry carotenoids (yellow pigment) which amounts vary according

to their diet (PATHARE; OPARA; AL-SAID, 2013). Thus, their diet may have provided them with a considerable amount of carotenoids.

It was noticeable that for all the drying techniques, the brightness of YM decreased after the process. According to Caivano and Del Pilar Buera (2012), the water of fresh tissues presents a different refraction index than the air of dry tissues; the reduction of water (moisture) during the drying may have affected the brightness (L*), making dried YM appear less bright than the fresh ones.

The redness of YM presented no significant variations between the pretreatments and drying techniques. Even though all statistically equal, samples dried by KMFD presented the highest values of redness.

KMFD samples presented higher darkness and redness than CVD ones, probably because in the CVD the vacuum was maintained during the whole drying, while in the KMFD samples were taken to a higher temperature (60 °C) at atmospheric pressure before applying the vacuum pulse; the cycle was repeated five times before they were maintained under constant vacuum. Additionally, for each drying technique, the value of brightness was higher for the blanched-dried YM than for the rinsed-dried ones. A possible explanation for that result can be that blanching before drying must have lessened browning reactions in YM (PATHARE; OPARA; AL-SAID, 2013).

The yellowness of YM decreased after drying, and for this parameter, the values of blanched YM decreased more than that of the rinsed ones. It can be interpreted that blanching, must have a greater effect of leaching YM carotenoids than rinsing, and could be the cause for the reduction of the yellowness.

For the total color changes (ΔE), the values of blanched-dried YM were higher than that of the rinsed-dried ones for all the drying techniques, the same range of ΔE was observed by Kröncke et al. (2019), that dried YM by oven drying and freeze-drying.

5 **CONCLUSION**

Conductive vacuum drying (CVD), conductive multi-flash drying I (KMFD) and an conductive multi-flash drying II (DVD) can produce dried *Tenebrio molitor* (YM) with low moisture content and water activity in short drying times. These three techniques, coupled with YM blanching, can be used for the reduction of YM's initial microbial loads. YM can be intentionally contaminated with *E. coli* in a challenge test and the combination of blanching plus CVD is effective in reducing *E. coli* loads. Higher total color changes are provoked by blanching before drying, but in compensation, it preserves more the initial brightness. From the perspective of drying times, microbial reduction and color change, CVD, DVD and KMFD could be alternatives to freeze-drying, oven or fluidized bed drying techniques for YM. In general, an only 15 s blanching of YM before drying leads to shorter drying times, higher brightness and more significant microbial reduction, making YM microbiologically safe for food and feed production. The drying techniques used in this study could be used in future studies, using different conditions of pressure and temperature, for example.

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