Ciência Rural, Santa Maria, v.54:04, e20230038, 2024
http://doi.org/10.1590/0103-8478cr20230038
ISSNe 1678-4596
FOOD TECHNOLOGY

Antimicrobial potential produced by *Hansenula wingei* and its use in mechanically deboned chicken meat

Bruno Seben de Almeida[1] Andressa Miranda[1] Alexandre Rodrigo Coelho[1] Alessandra Machado-Lunkes[1] Pedro Henrique Freitas Cardines[2] Roberta Barreiros de Souza[1] Mayka Reghiany Pedrão[1*]

[1]Departamento de Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná (UTFPR), 86020-430, Londrina, PR, Brasil. E-mail: maykapedrao@utfpr.edu.br. *Corresponding author.
[2]Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina (UEL), Londrina, PR, Brasil.

ABSTRACT: The domestic and international market is increasingly seeking for foods with reduced chemical additives. The viability using a versatile product as mechanically separate meat (CMS) to produce safety food is necessary to expand the product market. *Hansenula wingei* strain was selected, a yeast known as a killer toxin for poultry CMs application. The first part of this study consisted of defining the best growth condition for yeast, static or agitation, as well as the time either growth. The result obtained was the medium without agitation and 108 hours of growth at room temperature. The extract was subjected to the atomization drying process and the pulverized extract obtained was evaluated in vitro for minimum inhibitory concentration (MIC) tests for *Salmonella* sp, *Staphylococcus* positive coagulase, *E. coli* and mesophilic bacteria. Proximal composition, which the most relevant values to be analyzed were directed to total proteins that were 4.26g% (± 0.66) and 4.37g% (± 0.01) for agitation and unrestrained system, respectively. In the result of MIC, dry extracts were efficient in controlling the growth of all tested bacteria, including *Salmonella* spp. In the in situ tests, in which mechanically separated meat was raw materials to determine the antimicrobial factor action against food pathogens the concentration of 0.083g/ml of dry extract in its raw form obtained an inhibition factor as satisfactory as healing salts traditionally applied by the carneous industry. The viability of its growth and concentration for drying, can be considered a viable antimicrobial with good prospects for bio-conservative action.
Key words: bioconservative, antimicrobial, toxin killer, biocontrol, foodborne pathogens, poultry meat.

Potencial antimicrobiano produzido por *Hansenula wingei* em carne de frango mecanicamente separada

RESUMO: O mercado nacional e internacional busca cada vez mais por alimentos com reduzido teor de aditivos químicos. Estudar a viabilidade de um novo método de conservação natural de um produto tão versátil como a carne mecanicamente separada (CMS) se faz necessário para expandir o mercado do produto, seguindo as tendências das demandas alimentares dos consumidores atuais. Portanto foi selecionada uma cepa de *Hansenula wingei*, uma levedura conhecida pela produção de toxina killer para aplicação em CMS de aves. A primeira parte deste estudo consistiu em definir qual a melhor condição de crescimento para a levedura, consistindo este em ser estático ou em agitação, bem como o tempo no qual haveria o maior pico de crescimento da mesma. O resultado obtido foi o meio sem agitação e com 108 horas de crescimento a temperatura ambiente. Na sequência o extrato foi submetido ao processo de secagem por atomização e o pó obtido foi avaliado *in vitro* para testes de concentração mínima inibitória (MIC) para *Salmonella* sp, *Staphylococcus* coagulase positivo, *E. coli* e Mesofilos aeróbicos e definição de sua composição proximal, a qual os valores mais relevantes a serem analisados eram direcionados para proteínas totais que foram 4.26g% (±0.66) e 4.37g% (±0.01) para sistema com agitação e sem agitação, respectivamente. Como resultado de MIC, os extratos secos mostraram-se eficientes no controle do crescimento de todas as bactérias testadas, inclusive *Salmonella* spp. Nos testes *in situ*, no qual a carne mecanicamente separada foi a matéria prima testada para determinar a ação fator antimicrobiano contra patógenos de alimentos a concentração de 0.083g/ml do extrato seco, em sua forma bruta, obteve um fator de inibição tão satisfatório quanto aos sais de cura aplicados tradicionalmente pela indústria cárnea e pela viabilidade de seu crescimento e concentração por secagem, pode ser considerado um antimicrobiano viável e com boas perspectivas para ação bioconservante.
Palavras-chave: bioconservador, antimicrobiano, toxina killer, biocontrole. patógenos alimentares, carne de aves.

INTRODUCTION

Chicken meat is highly appreciated in Brazil and worldwide. It is mainly due to its high nutritional quality, wide availability, better cost-effectiveness compared with other animals. In addition to its flavor and versatility of consumption chicken meat has reduced religious restrictions. Preparation is much appreciated in cooking and in the food industry (EMBRAPA, 2022). This food is present in the daily diet of Brazilian people with its per capita consumption in 2021 leading to 45 kg/inhabitant (ABPA, 2022).

The Brazilian poultry sector has expanded a lot following the global trend of a gradual increase in the consumption of chicken meat. Its economic relevance is undeniable since Brazil is the second-largest producer of chicken in the world, behind only the United States, with more than 14 million tons produced in 2021, of which

Received 01.24.23 Approved 06.23.23 Returned by the author 08.19.23
CR-2023-0038.R2
Editors: Rudi Weiblen Rubén Domínguez

a third was exported, generating a national income of more than 7.5 million dollars (EMBRAPA, 2022).

According to the OECD-FAO (2022) Agricultural Outlook relative to market projections, global poultry meat consumption is expected to increase by 16% by 2031, which means that poultry farming will continue to be the main driver of poultry production growth meat. On a per capita basis, these robust growth rates in poultry consumption reflect the significant role it plays in the national diets of several populous developing countries, including China, India, Indonesia, Nigeria, Egypt, South Africa, Malaysia, Pakistan, Peru, the Philippines and Vietnam.

Among several ways to market chicken meat, mechanically deboned meat (MDM) has been widely spread due to its inexpensive cost, nutritional value and is used as a potential sustainable source of meat protein for food applications (PAGLARINI et al., 2022). Furthermore, MDM is easy to obtain and industrialized products such as emulsified and restructured meat products (e.g.; sausages, hamburgers, and nuggets), maintaining flavor and being useful for home cooking (PAP et al. 2022).

The international market is strict upon Brazilian poultry meat, imposing different standards for its import, mainly in the Middle East. Among the most common requirements are distinctive slaughter practices (e.g., halal – geared towards folks with Islamic religious affiliation), reduced microbial loads, and the use of chemical additives (UBABEF, 2013). Thus, many Brazilian industries have been trying to adapt to this reality to stay competitive.

To reduce microbial load, in addition to Good Manufacturing Practices (GMPs), the food industry can make use antimicrobials if it does not incur irregularities in the process (BRASIL, 2000). Meat quality monitoring methods are defined by Food and Agriculture Organization (FAO) and include good hygiene and manufacturing practices (GHPS, GMPs), HACCP and other rules and food safety regulations. Despite the constant concerns, the microbiological safety guarantee systems must receive more attention in different countries, being the interest in the food degradation important as it leads with disease development and may result in serious damages to human health (FAO, 2019). Temperature is one of the critical factors that may limit the deterioration of meat and meat products during processing, transportation and storage. It is known that increased temperature promotes the growth of bacteria, enzymatic activities that lead to economic losses. The deterioration of meat can be controlled by decreasing the temperature to 4 °C soon after slaughter for storage, and this is also the case for processed meat products effectively controlled by rapid freezing due to the development of microcrystals (REBEZOV et al., 2021).

According to BENSID et al. (2022), antimicrobials are substances of natural, semisynthetic, or synthetic origin that may be naturally present in food or may be added to delay or prevent the proliferation and growth of spoilage and pathogenic microorganisms (bacteria, yeasts, and, molds), and thus ensure food safety and quality. However, consumers have increasingly demanded high-quality foods with fresh flavor, minimally processed, and using fewer chemical preservatives. Therefore, to meet this demand, bio-preservatives has been investigated and shown potential to provide effective antimicrobial activity while reducing negative effects to health (YU et al., 2021).

Bioconservation or biocontrol refers to the controlled use of microbiota or its antimicrobial product to extend the shelf life of a product or ensure its safety (CALAZANS et al., 2021). This field of biotechnology has already made several advances in recent decades. Among these are the natural antimicrobials widely used in the industry, such as Nisin, a bacteriocin used to preserve dairy products, lact acid bacteria (STRACK et al., 2020). In agriculture and food industries, the interest is to apply yeast killer toxins as bioprotective agents which prevent the growth of competing microorganisms (STAROVIČ, 2020).

Killer toxins are one of these antimicrobial products used as biopreservatives. They are extracellular proteins produced by yeasts that can inhibit the growth of other sensitive microorganisms (FERREIRA, 2019). Yeasts with a killer factor have been extensively applied in vivo to control fungal growth in vegetables and fermentative musts, including those of the genus *Hansenula* (FONTANA et al. 2017). Given the growth in research and market needs, we decided to use a strain of *Hansenula wingei* (Hw) to produce antimicrobial and analyze its characteristics, as well as its potential application in dry form as a biopreservative in poultry MDM.

Thus, this study analyzed physicochemically a fermentation product of *Hw*, determining its antimicrobial efficacy after the drying process and its potential as a biopreservative in poultry mechanically deboned meat (MDM), aiming to broaden the scientific debate on biocontrol in food.

MATERIALS AND METHODS

From a solid culture of *Hansenula wingei* CMRP4947 (Hw) on potato dextrose agar (PDA), a

pre-inoculum was standardized in McFarland Scale 1 - about $3.0x10^7$ yeasts/mL, from which an aliquot of 100µL was transferred to six 1L flasks with yeast medium broth. Afterward, a 3L aliquot was transferred to 500mL erlenmeyer flasks and incubated under agitation at 110rpm in a shaker, and the remaining 3L statically, both at 25ºC for 148 hours. During incubation, a 3 mL aliquot of each growth condition (stirred and static) was aseptically collected, and these samples were subjected to absorbance reading by spectrophotometry at 600nm, 640nm, and 660nm. The readings were taken at 0, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 148 hours.

After 148 hours incubation time, the crude extract of each growth condition (stirred and static) was centrifuged at 10,000 rpm for 10 minutes, and the supernatant separated from the precipitate. Then, the supernatant was filtered through 0.44-µm-mesh membranes using a vacuum pump, while the precipitate was discarded. The crude extract was then dried by spray-dryer atomization, following: a feed flow rate of 0.7L/h, inlet temperature of 112 °C, air flow of 1.93m³/h, and 0.7mm nozzle.

The proximal composition of the dry extract was determined by analyzing total carbohydrates by a phenol-sulfuric method adapted from DUBOIS et al. (1956), total proteins by the biuret method of GORNALL et al. (1949), moisture by the infrared method of INSTITUTO ADOLFO LUTZ (1985), and fixed mineral residue (ash) by 550 °C, with the results expressed as g/100 g (AOAC, 2000). Content of lipids, in turn, was determined by difference.

The effectiveness of the killer toxin after concentration by drying process was ensured by tests of minimum inhibitory concentration (MIC) against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* coagulase positive strains, using Hw dry extract diluted at a concentration of 0.25g/mL. As a negative control, 1.5% lactic acid was used. These microorganisms were previously isolated from the poultry slaughter line where the MDM analyzed in this study is produced.

For MIC determination, the test bacterias were activated in BHI broth (38 °C/24-h) and inoculated in Mueller Hinton (MH) medium plates for 24-h additional growth and adaptation to the test medium. From this plate, colonies were diluted in MH broth at 0.5 McFarland scale ($1.5x10^8$ CFU/mL), and diluted again 100x for inoculum standardization to $1.5x10^6$ CFU/mL.

Each well of a 96-well microplate was added with 100µL MH broth and, except for control and also 100µL test bacteria ($1.5x10^5$ CFU). Six aliquots of diluted extract were also added, namely: 50, 60, 70, 80, 90, and 100µL, which thus encompassed six concentrations of dry extract to be tested. The process determined the lowest concentration to fully inhibit the microorganisms tested. The concentrations comprised: 0.041g/mL, 0.05g/mL, 0.058g/mL, 0.066g/mL, 0.075g/mL, and 0.083g/mL.

All wells received a total of 300µL containing MH medium, inoculum, and extract. Bacterium were added to collum wells in triplicates. Increasing concentrations of dry extract of *Hw* were added to the rows wells at the following proportions: 0, 50, 60, 70, 80, 90, and 100µL, plus control in added with lactic acid at 1.5% and without inoculum. Microplates were incubated at 37C/24H, followed an increase in the turbidity of the solution was observed, directly related to cell growth.

Chicken MDM was provided by a local abattoir and was previously analyzed in its quality control laboratory, following the current legislation (BRASIL, 2000). The analyses followed the guidelines of the seventh edition of the FDA Food Code (2022). For *Salmonella* spp., *Staphylococcus* coagulase-positive, as well as aerobic mesophilic microorganisms, all the results were within the standards. This preliminary analysis aimed to rule out potential cross-contamination or the influence of existing microorganisms.

Once received in the UTFPR laboratory, the MDM sample was divided into thirty-six 25-g portions, packed in sterile plastic bags. These sub-samples were identified according to the inoculum, they would receive and their freezing time. Twelve out of the 36 samples were inoculated with an aliquot (previously standardized at $1.5x10^5$ CFU/g) of *Staphylococcus* coagulase-positive, 12 with *E. coli* (representing aerobic mesophilic microorganisms), and 12 with *Salmonella* spp. Among the bacterial inoculated samples, three received a water and sodium nitrate solution at 0.03g/100g, which is the standard (P) to simulate the incorporation of curing salts into meat products in which MDM is used. Another six samples were inoculated with *Hw* dry extract, three under static (*Hw*1) and three under stirred (*Hw*2) systems. Samples under both conditions were diluted in sterile water (1:1), and the inoculum concentration in the extract was set to 83mg/g of MDM. Finally, the last three samples received no inoculum or treatment, making up the control (C).

MDM samples were frozen at -8 °C for up to 90 days, as required by MDM legislation (BRASIL, 2000). After 30, 60 and 90 days, 12 samples were removed for analysis, one of each

treatment. Analyses were adapted from the following methods: Plate count method APHA 39.63:2015 for *Staphylococcus* coagulase-positive, Plate count method APHA 08:2015 for aerobic mesophilic and ISO 6579 method for presence/absence of *Salmonella* spp in foods.

Statistical analysis was performed using the Biostat program (free software) in which quantitative descriptive analysis was carried out, and T test.

RESULTS AND DISCUSSION

The results of spectrophotometric absorbance indicated turbidity in the medium due to yeast growth, i.e., the higher the absorbance is, the higher the cell concentration is. Figure 1 shows the difference between a sample incubated under agitation and a sample in a static condition. The higher absorbance in samples under agitation from the first 24 hours indicates greater turbidity, hence greater cell growth. In figure 1, it becomes clear the difference in absorbance readings between static (600E, 640E, 660E) and stirred (600A, 640A, 660A) systems. Yeasts growth under agitation, as seen by the medium turbidity, is about twice that of yeasts under the static system. This result corroborated the data that *Hw* is a restrictively aerobic yeast. Therefore, under agitation, oxygen becomes more available to cells, increasing numerical growth. The analyses highlighted that the 600-nm wavelength was the best to determine *Hw* growth, both for static and agitation systems.

Based on extract weight, average yield of 0.55% for crude extract under the static system and 1.13% for crude extract under agitation. Table 1 shows the results of physical-chemical analyses. Significant differences between the means of growth systems were verified by the t-test.

Proximal composition results raised some questions. Differences in extract compositions do not account for the difference in drying efficiency, with the extract under agitation showing twice the yield. The main difference is related to carbohydrate and moisture contents. However, protein content, which is the key point of this study, showed no significant difference between both growth systems.

Ash contents agreed with the amounts of ammonium sulfate and sodium chloride inserted in the yeast culture medium. High ash contents (Table 1) resulted in high concentrations of salts since the medium itself was rich in sodium sulfate, ammonium sulfate, and sodium chloride.

It was not possible to detect total lipids in the samples. The results were not conclusive in different methodologies tested. In this sense, more sensitive methods should be tested for analysis of

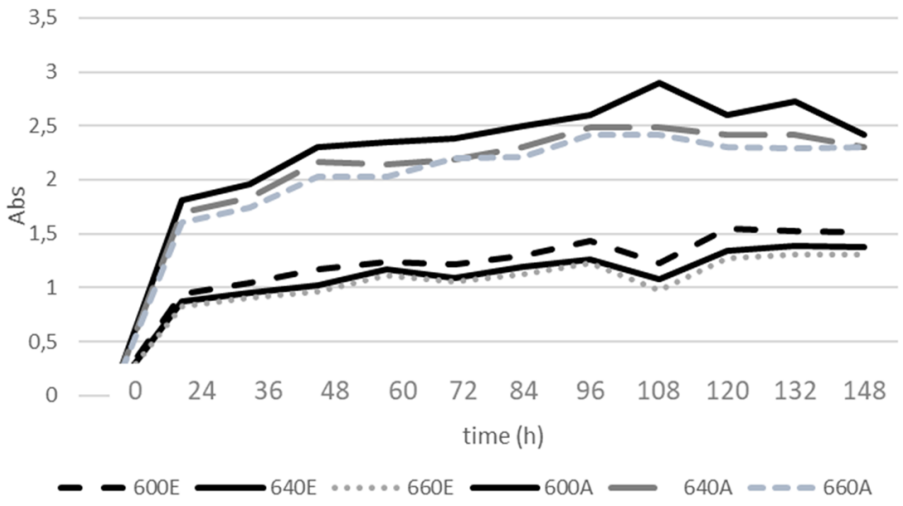

Figure 1 - Growth curve of the yeast *Hansenula wingei* under static conditions and with agitation using the same culture medium and turbidity (absorbance) indicating its growth performed at different wavelengths (600 a 660 nm).
Growth under agitation (A) and static (E) systems.

Table 1 - Proximal (partial) composition of dry extracts of *Hansenula wingei* submitted to two growth conditions, with agitation and static.

	----------Agitation (%)----------	----------Static (%)----------
Carbohydrates	5.72 (±0.31)[a]	4.4 (±0.7)[b]
Proteins	4.26 (±0.66)b	4.37 (±0.01)a
Moister	10.45 (±0.35)[b]	12.65 (±0.35)[a]
Ashes	49.6 (±1.7)a	48.72 (±0.32)a

a, b - Different lowercase letters in the same line (Tukey Test) indicate that samples are different from each other. (±) indicates standard deviation of the analyzes performed.

carbohydrates and proteins, once the methods used in this study may not have been the most suitable for the dry extract of *Hw*.

TAN et al. (2018) obtained for total protein from Metschnikowia saccharicola value 4.5g/100ml. RAGAVAN & DAS (2020) obtained values ranging from 0.56 to 48mg/100ml of total protein. Both authors working with purification of the killer fractions. Specifically in the material used in this research, as dry extract product of the fermentative process, a total of 4.26g% and 4.37g% for HW growth with and without agitation, respectively (Table 1)

From antagonism tests (MIC), observation of the turbidity of the wells, and indicated that the less cloudy, the greater the inhibition of bacteria tests. Different results were obtained between the dry extract under agitation and compared to the static system. When was tested the extract under agitation there was an inhibition of pathogen strains (*Salmonella* spp.) At the highest tested concentration of 0.083g/ml. While on the plate that dry extract was used in the static system, there was inhibition of the strains at smaller concentrations, such as *Salmonella* spp. at 0.066g/ml, and there was also inhibition of *E. coli* strain and partial inhibition of positive coagulase *Staphylococcus*. The defined MIC, from these observations was 0.083g/ml, the highest tested concentration, but even knowing the protein content (4-6 %) of the extract, the content of the killer toxin, is still unknown, for which detailed tests should be performed in the future.

The concentrations used in the dry extract of Hw used in this research as numerical values with purified toxins are compared to that used in research carried out by RAGAVAN & DAS (2020) of probiotic yeast strains *Yarrowia lipolytica* VIT-MN01, *Kluyveromyces lactis* VIT- Killer toxin producing MN02, *Lipomyces starkeyi* VIT-MN03, *Saccharomycopsis fibuligera* VIT-MN04 and *Brettanomyces custersianus* VIT-MN05. Among the five yeasts, three strains showed higher production of killer toxin. The maximum activity was obtained at concentration of 12 aU/mg against *Saccharomyces cerevisiae* strain, The results suggested that the killer toxin by these probiotics can be used as an antimicrobial agent to control microbial contamination in the food industry. *Metschnikowia pulcherrima* yeast strains have strong biocontrol activity against several microorganisms.

In a study carried out by BEDIR & KULEASAN (2021) specifically with purified killer toxin from this yeast, at concentrations of 1% and 2% in the formulations of beef meat balls, they obtained positive results for the control of microbial growth for total mesophilic aerobic bacteria, total aerobic psychrotrophic bacteria, coliform bacteria and *Staphylococci/Micrococci*. Conversely, RAGAVAN & DAS (2020) needed 50µL purified toxin killer to control *E. coli* development, as it can be seen from these two groups cited, the concentrations of antimicrobials used, even starting from purified products, vary considerably, which is justified since the active principles are different in their mechanisms of action.

In research presented by RAGAVAN & DAS (2020) with purified killer protein, they obtained protease activity indicating that there was inhibition of bacterial growth, although they do not specifically indicate in this article which killer toxin was obtained. The results suggested that yeast killer activity can be chosen to control gram positive and gram negative bacteria. The great difference between the methodologies of RAGAVAN & DAS (2020) and this research group is that purification was not carried out to obtain the killer toxin, so the crude dry extract from the fermentation process was used, being a preliminary study of a potential antimicrobial action. ÇORBACI & UÇAR (2017) studied a killer toxin

from *Debaryomyces hansenii* strains; however, they didn't use purification processes of the fermented material, that is, they developed the research with the crude extract, and obtained positive results for the studied antimicrobial activity.

The antagonism (MIC) tests are concluded, the tests directly began in the MDM, for which they defined specific potential inactivation of *Staphylococcus* coagulase-positive and mesophyls in the presence of dry HW extract obtained from the HW1 and HW2 growth means during 90 days of freezing, that is, what is approved by the Brazilian legislation for MDM.

Table 2 displays the results of inoculated MDM microbiological analyses. The control is inoculated samples with test microorganism, but without treatment with HW extracts. Standard refers to samples inoculated and treated with curing salt. And, *Hw*1 and *Hw*2 refer to samples inoculated and treated with dry extract of Hw from static and stirred systems, respectively.

The absence of typical *Salmonella* sp. colonies in selective and differential culture medium is related to the freezing temperature, as their growth is inhibited even in ranges considered as low as 2 °C, but hardly at freezing temperatures (BONNET et al., 2020). Mesophiles grew more expressively than did typical *Staphylococcus* colonies. This difference may be largely due to the known cold shock proteins in *E. coli* (ZHOU et al., 2021; PHADTARE, 2004). The antimicrobial action of Hw dry extract was more evident through counts of aerobic mesophiles (Table 2).

All the treatments differed for microbial counts, mainly at 30 days. The differences between counts at 30 and 60 days were mostly due to the cell death of microorganisms that could not survive under freezing temperature for long. Counts at 30 days and that of control were below the inoculum, the former being $1.5x10^5$ and the latter $2.3x10^4$. This also points to the negative effect of freezing on bacteria, mainly due to cell lysis by the formation of water crystals (MADIGAN et al., 2019). Differences were observed between treated and control samples, as well as between those treated with Hw dry extract from the static system and the other treatments. Based on that, we may conclude that the dry extract from static fermentation of *Hw* had better antimicrobial action than the curing salts traditionally used by the meat industry. Thus, our finding imperatively reinforces the potential of this extract to be used as a biopreservative in MDM.

Table 3 compares the inhibition factor of *Hw* dry extracts with the standard used for curing salts, by comparing microbial counts of treatments with that of control. The use of cure salt in this experiment was a way of indicating the efficiency of HW extracts, since curing salt is notoriously known for its bacteriostatic and preservative action in the meat industry (USDA, 2020). The higher the percentage, the stronger the inhibition of microbial growth. For *Staphylococcus* counts, the standard treatment used in the industry had similar efficacy to that with dry *Hw* extract from the static system. For mesophilic counts, the same extract had much higher efficacy in all counts. Therefore, *Hw* dry extract from

Table 2 - Counts of colonies of microorganisms inoculated in poultry MDM (in CFU/g).

----------*Staphylococcus*----------			
	30 Days	60 Days	90 Days
Control	$1.2x10^4$	<10	<10
Standard	$3.5x10^2$	<10	<10
*Hw*1	$5x10^2$	<10	<10
*Hw*2	$3.5x10^3$	<10	<10
----------Mesophiles----------			
	30 Days	60 Days	90 Days
Control	$2.3x10^4$	$2.8x10^3$	$3.5x10^3$
Standard	$8.7x10^3$	$1.4x10^3$	$1.2x10^3$
*Hw*1	$2.9x10^3$	$8.2x10^2$	$6.9x10^2$
*Hw*2	$7.1x10^3$	$6.7x10^2$	$7.1x10^2$

Control = No treatment; Standard = curing salts treatment; *Hw*1 = treatment with static dry extract; *Hw*2 = treatment with agitation dry extract.

Table 3 - Microbial load reduction in poultry MDM during 90 days of freezing compared to Control (in %) treated with curing salt and dry extract of *Hansenula wingei.*

----------*Staphylococcus spp.*----------			
Treatment	30 days	60 days	90 days
Standard	97.1	< 0.5	< 0.5
Hw 1	95.8	< 0.5	< 0.5
Hw 2	70.83	< 0.5	< 0.5
----------Mesophiles----------			
Treatment	30 days	60 days	90 days
Standard	62.17	50.0	65.7
Hw 1	87.39	70.7	0.3
Hw 2	69.13	76.1	79.7

Standard = curing salts treatment; *Hw*1 = treatment with static dry extract; *Hw*2 = treatment with agitation dry extract.

the static system has an inhibiting action equal to or greater than that of curing salts traditionally used as antimicrobials in the meat industry.

Overall, when comparing our findings with the parameters expressed by JONES, et al (2011) for potential biopreservatives, dry *Hw* extract can be considered as: non-toxic and regulated, as it is produced by a GRAS-recognized strain; low cost, because growth medium, process, and drying used are cheap and raw material is easily found; effective at low concentrations, as both MIC value and MDM test proved to be effective at an 8.3% concentration; without medical applications, as no publications were found proposing *Hw* extract for medical uses. Lastly, the sensory impact of *Hw* dry extract use in foods and its effectiveness after a long-term cooling or freezing must be further evaluated.

CONCLUSION

It is feasible to dry the early broth from growth of *Hansenula wingei* through spray dryer in parameters in this study and its use as in vitro and in situ antimicrobial, and it presented as effective as the curing salts used by the meat industry. However, it is necessary to research more about the constitution of this possible killer factor and future studies about purification and further clarification of its structure.

ACKNOWLEDGMENTS

The authors would like to thank the Multiuser Laboratory of Federal University of Technology – Paraná - Câmpus Londrina - for the performed analyses. CNPq for granting a Productivity Scholarship in Technological Extension Level II to Dr MRP and CAPES.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or data interpretation in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

DATA AVAILABILITY

Data will be made available on request.

REFERENCES

ABPA - Associação Brasileira de Proteína Animal. **Relatório Anual de Atividades – Carnes**. 2022. Available from: <https://abpa-br.org/wp-content/uploads/2022/05/Relatorio-Anual-ABPA-2022-vf.pdf>. Accessed: Jan. 01, 2023.

AOAC INTERNATIONAL. **Official Methods of Analysis**, 17th ed., Methods 967.25-967.28, 978.24, 989.12, 991.13, 994.04, and 995.20. 2000, Gaithersburg, MD. Available from: <http://webpages.icav.up.pt/PTDC/CVT-NUT/4294/2012/AOAC%202000.pdf>. Accessed: Jan. 01, 2023.

BEDIR, T. B.; KULEAŞAN, H. A natural approach, the use of killer toxin produced by Metschnikowia pulcherrima in fresh ground beef patties for shelf life extention. **International Journal of Food Microbiology**, v.345, p.109154, 2021. Available from: <https://www.sciencedirect.com/science/article/pii/S0168160521001136?casa_

token=_4-cxy0kwe8AAAAA:ItH4ccdP60XOvyAsTvZt8QPjvfK78H2V2h_Fz9O1UWy8HSDVkVvy1hb992eNw_UQlKoZ-O6O-M>. Accessed: Dec. 01, 2022. doi: 10.1016/j.ijfoodmicro.2021.109154.

BENSID, A. et al. Antioxidant and antimicrobial preservatives: Properties, mechanism of action and applications in food–a review. **Critical Reviews in Food Science and Nutrition**, v.62, n.11, p.2985-3001, 2022. Available from: <https://www.tandfonline.com/doi/abs/10.1080/10408398.2020.1862046>. Accessed: Dec. 01, 2022. doi: 10.1080/10408398.2020.1862046.

BONNET, M. et al. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. **New microbes and new infections**, v.34, p.100622, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S2052297519301192>. Accessed: Jan. 01, 2023. doi: 10.1016/j.nmni.2019.100622.

BRASIL. Instrução Normativa nº 4 de 31 de março de 2000. **Regulamento Técnico para Fixação de Identidade e Qualidade de Carne Mecanicamente Separada (CMS) de Aves, Bovinos e Suínos**. Diário Oficial da União da República Federativa do Brasil. Brasília, DF 05 mai. 2000. Seção 1, p.6. Available from: <http://engetecno.com.br/port/legislacao/carnes_cms.htm>. Accessed: Apr. 27, 2021.

CALAZANS, G. F. et al. Antimicrobial activity of Wickerhamomyces anomalus mycocins against strains of *Staphylococcus aureus* isolated from meats. **Food Science and Technology**, v.41, p.388-394, 2021. Available from: <https://www.scielo.br/j/cta/a/sLVrgd5Q4h3tKcVJmF7SftN/?lang=en&format=html>. Accessed: Jan. 01, 2023. doi: 10.1590/fst.39319.

ÇORBACI, C.; UÇAR, F. B. Production and optimization of killer toxin in Debaryomyces hansenii strains. **Brazilian Archives of Biology and Technology**, v.60, 2017. Available from: <https://www.scielo.br/j/babt/a/kksq9TG8JvXQSYP9rr9rV8q>. Accessed: Nov. 01, 2022. doi: 10.1590/1678-4324-2017160339.

DUBOIS, M. et al. Colorimetric Method Form Determination of Sugars and Related Substaces. **Nature**, v.28, n.3, p.350-356, 1956. Available from: <https://pubs.acs.org/doi/pdf/10.1021/ac60111a017?casa_token=w_gsMosXWVYAAAAA:BTj6tvwFMhhGiTzs4P5Lt9VqYnN6IKWkIwHM3OXJKdeUf0BZm0V_bFzes6pavirrSRE3meGxFN7sPWyS>. Accessed: Oct. 01, 2022. doi: 10.1021/ac60111a017.

EMBRAPA. **Qualidade da carne: do campo à mesa**. 2022 Available from: <https://www.embrapa.br/qualidade-da-carne/carne-de-aves>. Accessed: Jan. 01, 2023.

FAO - Food and Agriculture Organization. **Technical Guidance Principles of Risk-Based Meat Inspection and Their Application**. 2019. Available from: <http://www.fao.org/3/ca5465en/CA5465EN.pdf>. Accessed: Nov. 10, 2022.

FDA - Food and Drug Administrations. **CODE of Federal Regulations**. Title 21. Section CFR 866.3550. 2022. Available from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=866.3550>. Accessed: Aug. 01, 2022.

FERREIRA, F. F. et al. Levedura antagonista no controle de fungos filamentosos deteriorantes de alimentos. **Higiene Alimentar**, p.2793-2797, 2019. Available from: <https://pesquisa.bvsalud.org/portal/resource/pt/vti-24535>. Accessed: Aug. 01, 2022.

FONTANA, H. Y. Y. et al. Purificação parcial de toxina killer de *Hansenula wingei* visando aplicação no controle do desenvolvimento de fungos filamentosos deteriorantes de alimentos, p.199 -218. In: **Tópicos em Ciências e Tecnologia de Alimentos: Resultados de Pesquisas Acadêmicas** – v. 3. São Paulo: Blucher, 2017.

GORNALL, A. G. et al. Determination of serum proteins by means of the Biuret reaction. **Journal of Biological Chemistry**, v.177, p.751- 766, 1949. Available from: <https://www.academia.edu/download/7522360/gornall_etal.pdf> Accessed: Aug. 01, 2022. doi: 10.2508/chikusan.67.382.

JONES, R. J et al. Identifying new protective cultures and culture components for food biopreservation. In: LACROIX, C. **Protective cultures antimicrobial metabolites and bacteriophages for food and beverage biopreservation**. Cambridge: Woodhead Publishing, p.3-26, 2011. Available from: <https://www.sciencedirect.com/science/article/abs/pii/B9781845696696500017> Accessed: Aug. 01, 2022. doi: 10.1533/9780857090522.1.3.

LUTZ, A. Normas analíticas do Instituto Adolfo Lutz. **Métodos químicos e físicos para análise de alimentos**, v.2, 1985.

MADIGAN, M. T. et al. **Brock Biology of Microorganisms**. New York: Pearson. 2019.

OECD-FAO. **Agricultural Outlook 2022-2031**, OECD Publishing, Paris, 2022. Available from: <https://doi.org/10.1787/f1b0b29c-en>. Accessed: Jan. 01, 2023.

PAGLARINI, C. S. et al. Effect of commercial plant extracts on the oxidative stability of mechanically deboned poultry meat during chilled storage. **Food Research International**, p.112358, 2022. Available from: https://www.sciencedirect.com/science/article/pii/S0963996922014168?casa_token=STyLFp8LBNwAAAAA:67-1f84NkLP_GtZQsCr1qQzdipd_hvwKuuAsjHs-AgsmUUWg9dZRCO80msKK69Z-zUjjz9_YXfI>. Accessed: Jan. 01, 2023. doi: 10.1016/j.foodres.2022.112358.

PAP, N. et al. Optimization of valorization of chicken MDCM to produce soluble protein and collagen peptides. **Applied Sciences**, v.12, n.3, 1327, 2022. Available from: <https://www.mdpi.com/2076-3417/12/3/1327>. Accessed: Dec. 01, 2022. doi: 10.3390/app12031327.

PHADTARE, S. Recent Developments in Bacterial Cold-Shock Response. **Current Issues in Molecular Biology**, v.6, p.125-136, 2004. Available from: <https://www.mdpi.com/1467-3045/6/2/10>. Accessed: Oct. 01, 2022. doi: 10.21775/cimb.006.125.

RAGAVAN, M. L; DAS, N. Production and purification of killer toxin from probiotic yeasts and its effect on foodborne pathogens. **Journal of microbiology, biotechnology and food sciences**, v.10, n.3, p.350-353, 2020. Available from: https://office2.jmbfs.org/index.php/JMBFS/article/download/3219/86>. Accessed: Jan. 01, 2023. doi: 10.15414/jmbfs.2020.10.3.350-353.

REBEZOV, M. et al. Novel techniques for microbiological safety in meat and fish industries. **Applied Sciences**, v.12, n.1, p.319, 2021. Available from: <https://www.mdpi.com/2076-3417/12/1/319>. Accessed: Dec. 01, 2022. doi: 10.3390/app12010319.

STAROVIČ, M. **Yeast killer factors**: Biology and relevance in food biotechnology. 2020. Available from: <http://hdl.handle.net/10261/233114>. Accessed: Nov. 01, 2022.

STRACK, L. et al. Food biopreservation using antimicrobials produced by lactic acid bacteria. **Research, Society and Development**, v.9, n.8, p.e998986666-e998986666, 2020. Available from: <https://rsdjournal.org/index.php/rsd/article/view/6666>. Accessed: Oct. 01, 2022. doi: 10.33448/rsd-v9i8.6666.

TAN, C. et al. Marine killer yeast Metschnikowia saccharicola active against pathogenic yeast in crab and an optimization of the toxin production. **African Journal of Biotechnology**, v.17, n.21, p.668-679, 2018. Available from: <https://academicjournals.org/journal/AJB/article-full-text-pdf/D4CB5FF57196.pdf>. Accessed: Oct. 01, 2022. doi: 10.5897/AJB2017.16378.

UBABEF – União Brasileira de Avicultura. The Importance of The HALAL Market for Brazil's Chicken Exports. Brazilian Poultry Magazine. **Brazilian Poultry Association UBABEF**, São Paulo, Brasil, n.2. p.7-10. 2013.

USDA - United States Department of Agriculture. Cured Meat and Poultry Product Operations 03/09/2020. **Processing and Labeling Course**. Available from: <https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/fplic-5a-cured-meat-and-poultry-operations.pdf>. Accessed: Feb. 01, 2023.

YU, H. H. et al. Application of natural preservatives for meat and meat products against food-borne pathogens and spoilage bacteria: A review. **Foods**, v.10, n.10, p.2418, 2021. Available from: <https://www.mdpi.com/2304-8158/10/10/2418>. Accessed: Feb. 01, 2023. doi: 10.3390/foods10102418.

ZHOU, Z. et al. A cold shock protein promotes high-temperature microbial growth through binding to diverse RNA species. **Cell Discovery**, v.7, n.1, p.15, 2021. Available from: <https://www.nature.com/articles/s41421-021-00246-5>. Accessed: Jan. 01, 2023. doi: 10.1038/s41421-021-00246-5.