



Keratin hydrolysate improves the production of commercially valuable metabolites

Ronivaldo Rodrigues da Silva¹

Received: 29 September 2023 / Accepted: 18 December 2023 / Published online: 5 January 2024

© The Author(s), under exclusive licence to Plant Science and Biodiversity Centre, Slovak Academy of Sciences (SAS), Institute of Zoology, Slovak Academy of Sciences (SAS), Institute of Molecular Biology, Slovak Academy of Sciences (SAS) 2024

Abstract

In the integration of the circular economy, keratin hydrolysis is essential for preventing keratin accumulation in the environment and offers opportunities for utilising the resultant protein hydrolysate. Keratin hydrolysate can serve as an alternative nitrogen source for microbial cultures, and thus promote cost-effective production of commercially valuable metabolites such as biofuels, vitamins, organic acids, pigments, and polysaccharides. Considering the amount of keratin waste generated annually, particularly chicken feathers, exploring alternative approaches for utilising keratin hydrolysate could stimulate further research.

Keywords Bacteria · Biofuel · Feather · Fungi · Peptides · Protein hydrolysate

Introduction

Advances in the global market, along with an increase in the number of industries in diverse sectors, have contributed to an increase in industrial waste generation. Among them, the animal production industry generates substantial amounts of organic waste that requires extensive treatment. In particular, attention has been paid to keratinous materials generated from wool production and, on a larger scale, from the meat industry in the form of hooves, horns, and chicken feathers (Silva 2018).

The compact conformation and high molecular stability of keratin render it a recalcitrant material. Incineration is not an ecologically attractive alternative, and the disposal of protein waste in landfills can lead to uncontrolled anaerobic degradation and the release of ammonia and hydrogen sulphide (de Menezes et al. 2021). In this context, keratin hydrolysis by chemical or biological approaches offers a means to prevent its accumulation in the environment, and

the resulting keratin hydrolysate can potentially be utilised for various purposes, including as a biofertiliser and supplement for animal feed (Silva 2018; de Menezes et al. 2021).

In this discussion, in addition to these widely reported applications, more attention will be paid to the use of keratin hydrolysate to improve the production of other commercially valuable compounds, including vitamins, glutathione, organic acids, butanol, pigments (prodigiosin), and polysaccharides (xanthan gum). In 2020, the global production of the major types of keratin waste (wool and feathers from geese, guinea fowl, ducks, turkeys, and chickens) was estimated to be approximately 12 million tons (Chen et al. 2022). The keratinous material derived from this waste could serve as an inexpensive supplement to microbial growth media to obtain products of high commercial value. Several studies have reported similar findings.

Implementation of hydrolysed keratin in culture medium

A comparison of sheep wool hydrolysate, commercial tryptone peptone, and protease peptone for the production of hyaluronic acid (HA) using *Streptococcus zooepidemicus* ATCC 35246 has been reported (Arslan and Aydoğan 2021). The bacterial culture containing sheep wool peptone exhibited the best HA production, followed by media with

✉ Ronivaldo Rodrigues da Silva
rds.roni@yahoo.com.br

¹ Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista “Júlio de Mesquita Filho”, R/ Cristóvão Colombo, 2265. Jd Nazareth, Ibilce-Unesp, São José do Rio Preto, São Paulo, Brazil

tryptone and protease peptone, yielding HA concentrations of 3.54, 2.58, and 2.47 g/L, respectively. In media containing molasses and chicken feather peptone (CFP), Ozdal and Kurbanoglu (2019) reported improved citric acid production by *Aspergillus niger*. Among diverse peptones (4 g/L), the highest amount of citric acid was detected in the culture medium containing CFP, followed by the media with casein and Bacto peptones, with yields of 45.7%, 30.1%, and 34.6%, respectively (Ozdal and Kurbanoglu 2019).

Chicken feather hydrolysate has been investigated as a nitrogen source for ethanol and butanol production. Butanol

production from chicken feather and wheat straw hydrolysates has been achieved using *Clostridium* species. *Clostridium beijerinckii* strain NCIMB 8052 produced 4.6 g/L of butanol (Branska et al. 2020). In a study involving *Saccharomyces cerevisiae*, Serna-Cock et al. (2018) found that despite the best ethanol production being achieved using media containing urea (58.12 g/L), chicken hydrolysate emerged as a suitable substitute for conventional nitrogen sources in fermentative media (46.91 g/L ethanol production). Therefore, keratin hydrolysate has been proposed as an alternative and an inexpensive nitrogen source for bio-fuel production.

Taskin (2013) reported improved glutathione (GSH) production by *S. cerevisiae* when chicken feather hydrolysate (20 g/L) was used as an amino acid source, achieving a maximum GSH production of 271 mg/L, followed by fish peptone (255 mg/L), tryptone-peptone (215 mg/L), and control culture (126 mg/L). Hassan et al. (2020) used donkey hair hydrolysate as an additive in the culture medium to produce vitamins B1, B2, and B12 using *S. cerevisiae* ATCC 64712. Table 1 summarises the metabolites produced using keratin hydrolysate.

Advantages and disadvantages of obtaining keratin hydrolysate

Recycling of keratin waste is in high demand owing to the large quantities of keratinous waste generated annually. Keratin hydrolysate is a valuable product derived from keratin hydrolysis using chemical (acid and alkaline) or biological (microbial/enzymatic) methods.

Chemical hydrolysis of keratin yields low-molecular-weight components; however, this approach is not recognised as an environmentally friendly treatment, as it requires high-molarity acid/alkaline solutions and wastewater neutralisation (Shestakova et al. 2021; de Menezes et al. 2021). Microbial hydrolysis depends on the selection of non-pathogenic keratinolytic microorganisms for keratin degradation. Furthermore, some of the produced keratin hydrolysate is consumed by microorganisms during growth. Complete keratin degradation via hydrolysis conducted using isolated keratinases (cell-free enzymes) remains a challenge (Shestakova et al. 2021; de Menezes et al. 2021; Menezes et al. 2023). However, the biological degradation of keratin is more sustainable than chemical treatments as it does not require the use of harmful chemicals (de Menezes et al. 2021; Shestakova et al. 2021).

Overall, keratin hydrolysis can contribute to solving environmental issues related to keratin-rich waste disposal and offers opportunities for the utilisation of the resulting hydrolysate. In addition to the commonly proposed

Table 1 Examples of metabolites produced using keratin hydrolysate as an additive in culture media for microorganisms

Microorganisms	Keratin sources	Keratin hydrolysis	Metabolites produced	References
<i>Saccharomyces cerevisiae</i> strain ATCC 64712	Donkey hair hydrolysate	Enzymatic hydrolysis	Vitamin B-complex	Hassan et al. (2020)
<i>Streptococcus zooepidemicus</i> ATCC 35246	Sheep wool hydrolysate	Chemical hydrolysis (acid and alkaline)	Hyaluronic acid	Arslan and Aydogan (2021)
<i>Aspergillus niger</i> MO-25	Chicken feather hydrolysate	Acid hydrolysis	Citric acid	Ozdal and Kurbanoglu (2019)
<i>Clostridium beijerinckii</i>	Chicken feather hydrolysate	Alkaline hydrolysis	Butanol	Branska et al. (2020)
<i>Lactobacillus reuteri</i> LHR14 and <i>L. casei</i> CCDM 198	Chicken feather hydrolysate	Alkaline hydrolysis	Lactic acid	Gharwalová et al. (2018)
<i>Rhizopus oryzae</i> TS-61	Chicken feather hydrolysate	Acid hydrolysis	Lactic acid	Taskin et al. (2012)
<i>Saccharomyces cerevisiae</i>	Chicken feather hydrolysate	Acid hydrolysis	Glutathione	Taskin (2013)
Recombinant <i>S. cerevisiae</i>	Chicken feather hydrolysate	Microbial culture (<i>Trichoderma atroviride</i> F6)	Glutathione	Qiu et al. (2014)
<i>Pseudomonas aeruginosa</i> OG1	Chicken feather hydrolysate	Acid hydrolysis	Rhamnolipid biosurfactant	Ozdal et al. (2017)
<i>Xanthomonas campestris</i> MO-03	Chicken feather hydrolysate	Acid hydrolysis	Xanthan gum	Ozdal and Kurbanoglu (2018)
<i>Serratia marcescens</i> MO-1	Ram horn peptone	Acid hydrolysis	Prodigiosin	Kurbanoglu et al. (2015)

applications, this discussion offers perspectives on the use of keratin hydrolysate as an inexpensive nitrogen source in microbial cultures for producing metabolites. This discussion may stimulate further investigations into the application of this animal biomass.

Declarations

Competing interests The author declares no competing financial interest.

References

- Arslan NP, Aydoğan MN (2021) Evaluation of Sheep wool protein hydrolysate and molasses as low-cost fermentation substrates for Hyaluronic Acid production by *Streptococcus zooepidemicus* ATCC 35246. *Waste Biomass Valori* 12:925–935. <https://doi.org/10.1007/s12649-020-01062-w>
- Branska B, Fořtová L, Dvořáková M, Liu H, Pataková P, Zhang J, Melzoch M (2020) Chicken feather and wheat straw hydrolysate for direct utilization in biobutanol production. *Renew Energy* 145:1941–1948. <https://doi.org/10.1016/j.renene.2019.07.094>
- Chen H, Gao S, Li Y, Xu H-J, Li W, Wang J, Zhang Y (2022) Valorization of livestock keratin waste: application in agricultural fields. *Int J Environ Res Public Health* 19:6681. <https://doi.org/10.3390/ijerph19116681>
- de Menezes CLA, Santos RC, Santos MV, Boscolo M, Da Silva R, Gomes E, Silva RR (2021) Industrial sustainability of microbial keratinases: production and potential applications. *World J Microbiol Biotechnol* 37. <https://doi.org/10.1007/s11274-021-03052-z>
- de Menezes CLA, Boscolo M, da Silva R, Gomes E, Silva RR (2023) The degradation of chicken feathers by *Ochrobactrum intermedium* results in antioxidant and metal chelating hydrolysates and proteolytic enzymes for staphylococcal biofilm dispersion. *3 Biotech* 13. <https://doi.org/10.1007/s13205-023-03619-7>
- Gharwalová L, Paulová L, Patáková P, Branská B, Melzoch K (2018) Use of wheat straw and chicken feather hydrolysates as a complete medium for lactic acid production. *Czech J Food Sci* 36:146–153
- Hassan MA, Taha TH, Hamad GM, Hashemd M, Alamri S, Mostafa YS (2020) Biochemical characterisation and application of keratinase from *Bacillus thuringiensis* MT1 to enable valorisation of hair wastes through biosynthesis of vitamin B-complex. *Int J Biol Macromol* 153:561–572. <https://doi.org/10.1016/j.ijbiomac.2020.03.032>
- Kurbanoglu EB, Ozdal M, Ozdal OG, Algur OF (2015) Enhanced production of prodigiosin by *Serratia marcescens* MO-1 using ram horn peptone. *Braz J Microbiol* 46:631–637. <https://doi.org/10.1590/S1517-838246246220131143>
- Ozdal M, Kurbanoglu EB (2018) Valorisation of chicken feathers for xanthan gum production using *Xanthomonas campestris* MO-03. *JGEB* 16:259–263. <https://doi.org/10.1016/j.jgeb.2018.07.005>
- Ozdal M, Kurbanoglu EB (2019) Citric acid production by *Aspergillus Niger* from Agro-industrial By-Products: molasses and chicken feather peptone. *Waste Biomass Valori* 10:631–640. <https://doi.org/10.1007/s12649-018-0240-y>
- Ozdal M, Gurkok S, Ozdal OG (2017a) Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone. *3 Biotech* 7:117. <https://doi.org/10.1007/s13205-017-0774-x>
- Qiu Z, Tan H, Zhou S, Cao L (2014) Surface Display of a Bifunctional glutathione synthetase on *Saccharomyces cerevisiae* for converting chicken feather hydrolysate into glutathione. *Mol Biotechnol* 56:726–730. <https://doi.org/10.1007/s12033-014-9750-4>
- Serna-Cock L, Rengifo-Guerrero CA, Rojas-Restrepo MA (2018) Use of Earthworm (*Eisenia foetida*) Flour and Hydrolyzed Chicken Feathers as sources of Nitrogen and minerals for ethanol production. *Waste Biomass Valori* 9:1513–1522. <https://doi.org/10.1007/s12649-017-9931-z>
- Shestakova A, Timorshina S, Osmolovskiy A (2021) Biodegradation of keratin-rich husbandry waste as a path to sustainable agriculture. *Sustainability* 13:8691. <https://doi.org/10.3390/su13168691>
- Silva RR (2018) Keratinases as an alternative method designed to solve keratin disposal on the environment: its relevance on agricultural and environmental chemistry. *J Agric Food Chem* 66:7219–7221. <https://doi.org/10.1021/acs.jafc.8b03152>
- Taskin M (2013) A new strategy for improved glutathione production from *Saccharomyces cerevisiae*: use of cysteine- and glycine-rich chicken feather protein hydrolysate as a new cheap substrate. *J Sci Food Agric* 93:535–541. <https://doi.org/10.1002/jsfa.5818>
- Taskin M, Esim N, Ortucu S (2012) Efficient production of l-lactic acid from chicken feather protein hydrolysate and sugar beet molasses by the newly isolated *Rhizopus oryzae* TS-61. *Food Bioprod Process* 90:773–779

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.