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ORIGINAL ARTICLE

Valorization of Chicken Viscera as Natural Raw Material Source: Application to Hydrolysis of Fatty Esters for Sewage Treatment

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Abstract

"Chicken viscera" constitutes very abundant domestic wastes interestingly investigated in the present paper. The efficiency of this crude slaughter co-product of high protein component, as biocatalyst, for the hydrolysis of fatty acid esters was reported and that, without any pre-treatment. The crude chicken intestine powder (CIP) has shown a high reactivity for the hydrolysis of fatty esters. Two biocatalyst preparations were independently explored for the bioresolution of sec-phenyl alkyl carbinol esters: the CIP preparation and the crude chicken intestine acetone powder CIAP preparation. The last one has shown good catalytic activity during the bio-hydrolysis in biphasic medium. Furthermore, the direct hydrolysis of milk fat using CIAP (500 mg) reveals the elimination of fats present in 50 ml of treated milk. These results open up very interesting prospects for the use of this biowaste for the treatment of milk fat.

Keywords Chicken viscera · Biowastes valorization · Crude chicken intestines acetone powder · Fatty acid esters · Bio-hydrolysis · Sustainable chemistry

Statement of Novelty We have exploited a particularly interesting biomass, the "chicken viscera," in enzymatic catalysis, for the hydrolysis of fatty acid esters. The extraction procedure is simple and fits perfectly into a green chemistry process. Two extracts were tested. The extracts are reactive in enzymatic hydrolysis, and they are also effective in removing fat from fresh milk, which is particularly useful and valuable in the treatment of wastewater from dairies and oil mills.

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Introduction

Over the last 30 years, the worldwide supply of white meat has grown at an average rate of 5% per year [1]. In 2017, this sector produced 100 million tons all over the world [2]. The Food and Agriculture Organization of the United Nations (FAO) has reported that global chicken production has almost doubled over the last 25 years [3]. In this context, and as a result of growing awareness of the environmental impact of effluent organic matter [4], the valorization of those waste for the elaboration of new natural raw materials has been a pressing priority for the scientific community. Furthermore, the UN's sustainable development agenda has set vital targets to be achieved by 2030 to reducing wastes and protecting natural resources [5] including (i) the management and use of natural resources sustainably and efficiently and (ii) a significant reduction in the generation of waste through prevention, reduction, recycling, and reuse.

In Algeria, animal by-products are not recovered but disposed of [6], many of waste which are dumped or sold to informal sector recyclers who use them as animal feed [7]. The National Waste Agency (AND) was estimated the industrial wastes in Algeria at 2.5 million tons/year, while the recovery rate did not exceed the threshold of 7%. Unfortunately, until now, this last area still untapped [8]. Among the main objectives of sustainable development, reducing food wastes is a challenged task, either by avoiding its creation or by turning it into a resource.

The processing of live animal into a carcass generates noble products, essentially, muscle tissue compound (meat), as well as various co-products among offal, bones, organic matter wastes, etc. [9]. These biowastes are mainly buried in landfills and/or burned in incinerators, which contributes to environmental damage and disease transmission.

However, some byproducts of the poultry industry were advantageously used as renewable sources in various manufacturing applications. For example, chicken feathers are used for the production of textiles [10], bio-fertilizer [11], bioplastics [12], paper [13], or for animal feed [14]. Moreover, the extraction and characterization of functional proteins from offal such as liver [15], lung [16], heart [17], viscera [18], and bones [19] were reported.

Chicken viscera constitutes an important by-product of the meat industry. Owing to its composition of 92.5% organic matter, including 32.5% protein, 20% lipids, 40.1% carbohydrates, and 7.4% ash, seems a potential cheap biocatalyst candidate [20].

With the huge advancement of the industrial biotechnologies, the developments of new efficient and cheap biomaterials like biocatalysts still important. This is in order to develop sustainable, clean, and viable alternatives to hard chemical processes [21, 22]. Industrial-scale organic biotransformations, including hydrolysis, oxidation, reduction, addition/elimination, and transesterification reactions, were widely exploited in the pharmaceutical, chemical, food, cosmetics, and textile industries [23]. Hydrolases, microorganisms, or plants are also successfully used to access chiral building blocks through enzymatic catalysis [24–27]. Otherwise, fat enzymatic hydrolysis has strong industrial interest as oleochemical feedstock. It is a promising route for the valorization of this waste towards a circular economy [28].

The most studied mammalian lipases are lipases related to fat digestion and absorption. Rabbit gastric lipase has been studied in enzymatic catalysis. [29, 30] Toward chemically alike, but sterically nonequivalent ester groups within one single triglyceride molecule was investigated [31]. Our group studied these aspects [32, 33].

The present paper reports for the first time the valorization of a domestic biowastes "chicken viscera" as a potential biocatalyst source. Two preparation ways were described

and used for the bio-hydrolysis of fatty esters, direct treatment of fat milk, and the bioresolution of some racemic secondary esters of a high added value. Milk fats present in effluent pose many problems for the water treatment. We also examined the hydrolysis potential of this chicken intestine powder on fresh milk fat. At the best of our knowledge, only the chicken liver acetone powder prepared in lab was exploited for the resolution of racemic 1-phenylethanols and 1-phenylpropanols by the hydrolysis of their corresponding acetates [34] and for the hydrolysis of (±)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid esters [35].

Materials and Methods

Two biocatalyst preparations were explored from the whole chicken intestines. The preparation protocols are different, the first uses acetone during the extraction, while the second preparation is carried out without this solvent. The crude chicken intestine acetone powder (CIAP) was used for the biohydrolysis of fatty acid esters and examined for the kinetic bioresolution of 1-phenyl ethanol. The efficiency of the last preparation was compared to the crude chicken intestine powder (CIP), during the bioresolution of several esters. We have also used these extracts for the hydrolysis of milk fat for use in wastewater from the food industry.

CIAP Preparation Procedure

For the first preparation, the chicken intestines, freshly collected from a local slaughterhouse, were cleaned and washed very carefully several times (Fig. 1). They were then subjected to grinding for 5 min in a blender. The shredded material was rinsed in acetone 3 times, filtered, and dried in an oven at a temperature of 35–40 °C until a constant weight was attained. The dry mixture was then blended and the powder obtained, marked CIAP, was stored in a freezer at – 4 °C. From 1.5 kg of chicken viscera, 200 g of CIAP was obtained. The extract was tested in esterification and hydrolysis reactions.

CIP Preparation Procedure

Following the obtained results, we have envisaged to compare the first preparation with the native chicken intestine powder, by omitting the acetone-extraction step (Fig. 2). The chicken intestines came from the same slaughterhouse (BENMERABT) [36] and were

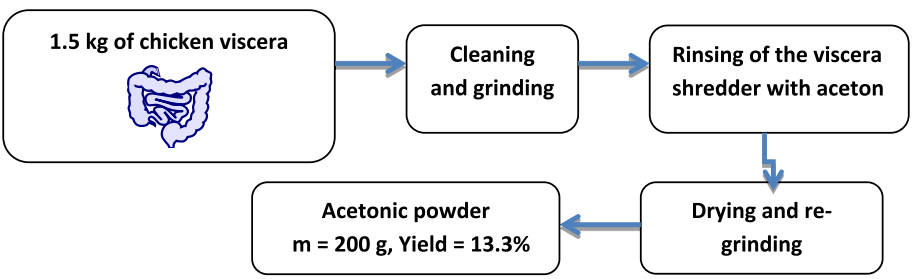


Fig. 1 Extraction procedure of CIAP

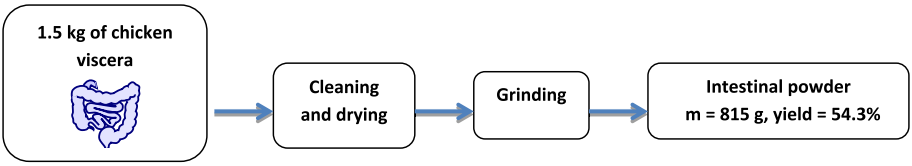


Fig. 2 Extraction procedure for CIP

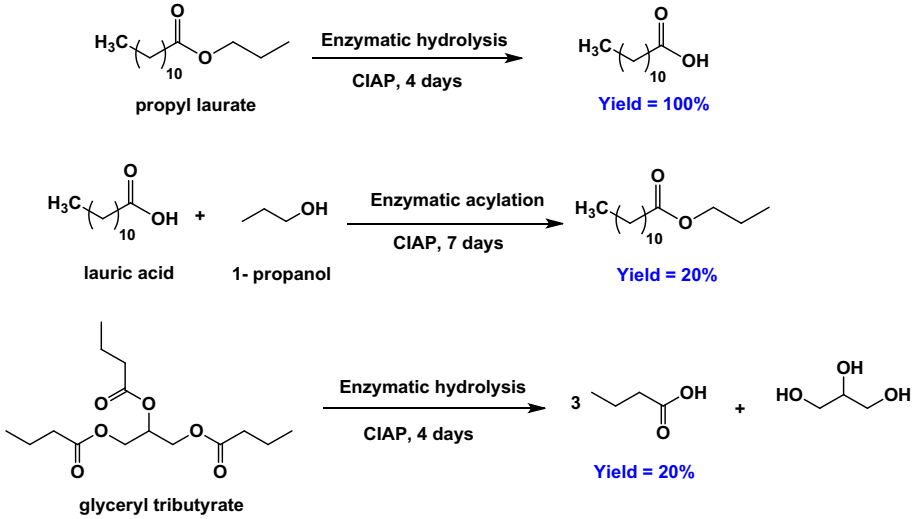
used directly after slaughter. They were thoroughly washed, cleaned, oven-dried at 50 °C to constant weight, and then ground. The powder obtained is kept in the freezer at – 4 °C until it is used. From 1.5 kg of chicken viscera, 815 g of CIP was obtained.

Results

Lipases is a superfamily of enzymes that share a characteristic α/β-hydrolase fold surrounding the catalytic triad where hydrolysis takes place [37, 38]. These are water-soluble enzymes hydrolyzing insoluble triglyceride substrates, and studies on these enzymes have led to the development of specific interfacial kinetic models. Structure–function studies on lipases have thrown light on the interfacial recognition sites present in the molecular structure of these enzymes, the conformational changes occurring in the presence of lipids and amphiphiles, and the stability of the enzymes present at interfaces. [39] We chose two extraction methods, the first using a solvent which made it possible to obtain CIAP, the second protocol does not introduce any solvent, CIP. The difference in extraction methods could potentially lead to variances in the conformation of the enzyme, particularly in its active site. The use of acetone in the extraction process for CIAP might introduce unique environmental conditions and interactions during the isolation of the enzyme, influencing its three-dimensional structure.

Esterification and Hydrolysis Reaction with CIAP

The activity of CIAP preparation has been checked during the esterification and hydrolysis reactions (Scheme 1). It is to be underlined that only the biohydrolysis was checked with the liver acetone powder of animals, including chicken [40, 41]. The obtained results seem very interesting and differ according to substrate nature. Total hydrolysis of propyl laurate was recorded after 96 h, while for the same reaction time the hydrolysis of glyceryl tris-butyrate did not exceed the threshold of 20% of yield. For the bioesterification of lauric acid by 1-propanol, the corresponding ester was recovered with 20% yield upon 7 days of stirring. These results showed a efficiency of CIAP preparation for the hydrolysis of fatty acid esters, which encouraged us to explore the efficiency of this biocatalyst for the treatment of FOGs (fat, oil, and grease) as a part of wastewater [42]. To better this purpose, we have envisaged to study its behavior directly on the milk fats.



Scheme 1 Esterification and hydrolysis reaction with CIAP

Hydrolysis of Milk Fats

Milk fats present in dairy effluent and pose many problems for the water treatment. To explore the potentiality of our cheap biocatalyst preparation for the remediation of this problematic, we have examined its behavior directly on fresh milk fat. A primary chemical test [43] was generally used to determine the fat content of the substance often in milk and cream. This procedure based on the release of the fat matter from the mixture by appropriate acid treatment; it is then separated and collected in a graduated column by centrifugation. The CIAP preparation was added to 50 ml of fresh milk and stirred at 25 °C for 24 h; then, the mixture was filtered under vacuum; then, the biocatalyst was recovered. The amounts of CIAP used were respectively: 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, and 1 g. The results were collected in Table 1.

It was found that the fat present in the milk was completely eliminated for a quantity of 500 mg of CIAP (Fig. 3). These results have led to very interesting prospects for the use of this biowaste for the treatment of dairy water and could be extended to FOGs' wastewater treatment.

CIAP Catalyzed Kinetic Resolution of 1-Phenyl Ethanol

In the continuity of our laboratory investigations on the use of kinetic resolution reactions catalyzed by lipases to access to enantiomerically enriched building blocks, for example, the fatty esters trough esterification and hydrolysis [44–46], we have scrutinized the enzymatic reactivity and selectivity of this newly developed biocatalyst. The kinetic resolution of sec-1-phenylethanol by acylation with succinic anhydride and by hydrolysis of the corresponding ester were selected [47–49].

Table 1 Treatment of milk fat by CIAP

Entry	Conditions[a]	Percentage of fat (%)[b]
1	Fresh milk	15
2	Milk after 24 h of stirring	15
3	Milk + 100 mg of CIAP	10
4	Milk + 200 mg of CIAP	9
5	Milk + 300 mg of CIAP	5
6	Milk + 400 mg of CIAP	3
7	Milk + 500 mg of CIAP	0
8	Milk + 1000 mg of CIAP	0

[a]50 ml of fresh milk with CIAP at 25 °C for 24 h

[b]Measured using the Geber method [35]

Enzymatic acylation was performed on 1 mmol of 1-phenyl ethanol with 1 mmol of succinic anhydride and 250 mg of CIAP in 6 mL of Et_2O for 4 days at 30 °C. Hydrolysis of racemic acetate is performed on 1 mmol of 1-phenylethyl acetate with 250 mg of CIAP t in a mixture of 2 ml ethyl ether and 8 ml K_2HPO_4/Na_2HPO_4 buffer solution pH = 7. Magnetic stirring was maintained for 4 days at 37 °C. The resulting mixture of the product and the residual substrate was filtered over celite and extracted with ethyl ether (Scheme 2).

As described on scheme 2, the CIAP has shown a high reactivity for the hydrolysis without selectivity (C = 71%, E = 1) compared to the acylation one. These results confirm those obtained above with the achiral substrates. Thus reveal the hydrolytic properties of this biocatalyst preparation, for better apprehend the mode of action of this biocatalyst, we have extended the study of the biohydrolysis onto a set esters. We have focused on the impact of the leaving group on the reactivity of CIAP (Table 2).

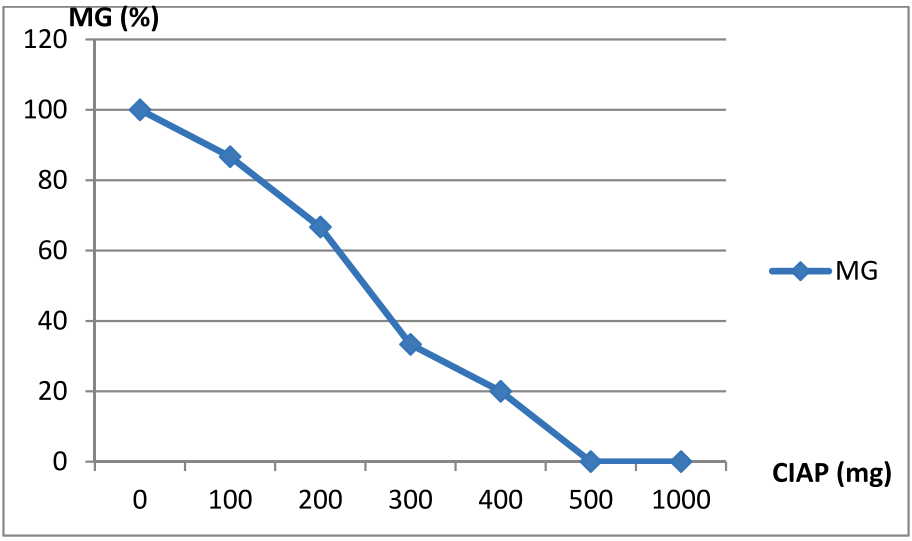
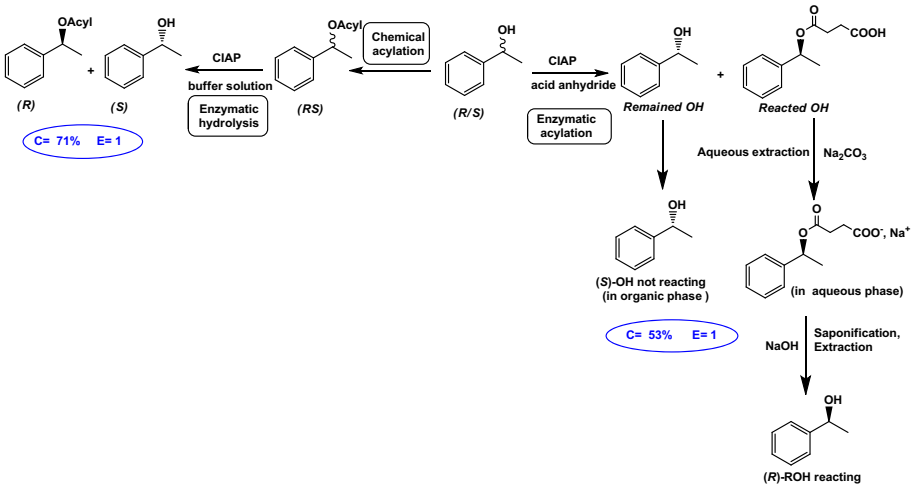


Fig. 3 Percentage of fatty matter in function of CIAP amount



Scheme 2 Kinetic resolution by acylation/hydrolysis with CIAP

The results summarized in Table 2 showed that during the hydrolysis of esters 1e-1f, the hindrance of the ester group strongly impact the reactivity of CIAP, the conversion decreases from C = 71.4% to C = 51.6%, and then to C = 39% when going from the methyl derivative, to phenyl and to tertiary butyl respectively. A drastic decrease of the CIAP reactivity was noted when using decanoate derivatives as substrate (Table 2, entry 4: Conv = 10%) and total activity inhibition was recorded the laurate ester. This loss of activity is probably due to the difficulty of reaching the active site of the enzyme when the size of the ester moiety increases. Changing the buffer solution K_2HPO_4/Na_2HPO_4 by a Tris–HCl solution of pH = 8 where a slight advancement was observed Conv = 5.7% (Table 2, Entry 6), indeed, this solution has labile protons and facilitates the penetration of the substrate into the active site of the enzyme [50–52].

Table 2 CIAP catalyzed Kinetic bioresolution of esters 1a-1e

Entry[a]	Substrat	ee_s[b] (Yield)[c] (%)	ee_p[b] (Yield)[c] (%)	C (%)[d]	E
1	*Rac-1a*	1.4 (57)	0.56 (24)	71.4	1
2	*Rac-1b*	0.16 (43)	0.15 (38)	51.6	1
3	*Rac-1c*	0.84 (55)	1.32 (26)	39	1
4	*Rac-1d*	0.44 (61)	3.88 (18)	10.2	1
5	*Rac-1e*	1 (92)	-	-	-
6	*Rac-1e* In the Tris–HCl	0.4 (83)	7 (12)	5.4	1

[a]1 mmol of racemic ester, in 2 ml diethyl ether and 8 ml buffer solution K_2HPO_4/Na_2HPO_4 at 37 °C for 96 h

[b]Enantiomeric excesses are measured by chiral chromatography, HPLC, or chiral GC

[c]Chemical yields are calculated after extraction

[d]Conversion rate: $C = ee_S/ee_P + ee_s$; selectivity factor $E = Ln[(1\text{-}C)(1\text{-}ee_s)]/Ln[(1\text{-}C)(1 + ee_s)]$ [51, 52]

Determination of the Protein Content by the Lowry Method

In order to establish the rate of advancement of the chemical reactions carried out with the two preparations, the amount of protein contained in each enzyme extract is assayed according to the method of Lowry et al. [53]. Protein concentrations were calculated by linear interpolation, using a standard range containing bovine serum albumin.

Using the calibration line, the protein concentration of the samples is calculated taking into account the dilutions used. The results of the quantitative protein assay study by the Lowry method (Table 3) showed that both extracts have the same protein mass which is 25 mg/g.

Application in the Hydrolysis of Esters 1a, 1f-1 h

At this stage of our study, we have performed the hydrolysis on other types of racemic benzyl esters of potential interest with both CIPs. The reaction was performed in a biphasic medium (diethyl ether/buffer solution pH = 7: v/v: 2/4) in the presence of 250 mg of CIAP or CIP for 1 mmol of the substrate. The residual acetate and alcohol formed are recovered after 96 h of reaction and separated on a silica gel chromatographic column. The enantiomeric excesses are determined by HPLC on a CHIRACEL OD-H chiral column. The results are shown in Table 4.

The results reported on Table 4 show that the selectivity of both preparations in the hydrolysis reaction for the majority of esters (1a, f–h) is variable $1 < E < 9$ with conversions between $4\% < C < 74\%$. Low selectivity ($E < 9$) was observed for derivatives 1a, 1f, 1 g, and 1 h upon the biohydrolysis. However, a higher hydrolytic activity of the CIAP compared to CIP was observed for these substrates, the conversion decreases from C = 71 to C = 26% for 1a (Table 4, entry 1 versus 2), from C = 65 to C = 53% for 1f (Table 4, entry 3 versus 4) and from C = 15% to total inactivity of CIP for the 1 g ester (Table 4, entry 5 versus 6). CIP is active for the hydrolysis of 1 h (C = 8%, E = 9) (Table 4, entry 8 versus 7) and selectivity of E = 9. Thus, the structure of the racemic esters significantly affects the reactivity and selectivity of both preparations. The shape and penetration of the substrates into the active site of the biocatalysts varied for both extracts and it was found that CIAP exhibited better catalytic activity than CIP even though the protein content of both preparations was similar. These results can be explained by taking into account the extraction procedure which probably impacts the shape of the active site in both extracts.

Table 3 Protein determination of CIAP and CIP by the Lowry method

	CIAP (20 mg raw/ml): 1/10	CIP (20 mg raw/ml): 1/10
Average absorbance	199 nm	198 nm
Protein concentration	0.51 mg/ml	0.492 mg/ml
Protein mass per raw sample mass	**25.5 mg/g**	**24.6 mg/g**

Table 4 Kinetic resolution by hydrolysis: comparison of CIAP and CIP

Entry[a]	Substrat	Biocat	ee_s[b] (Yield)[c] (%)	ee_p[b] (Yield) [c](%)	C (%)[d]	E[d]
1	***Rac-1a***	**CIAP**	1.4 (28)	0.56 (69)	71.4	1
2		**CIP**	**21 (67)**	**59.3 (21)**	**26.1**	**5**
3	***Rac-1f***	**CIAP**	24 (58)	13 (47)	65	2
4		**CIP**	**26 (43)**	**23 (49)**	**53**	**2**
5	***Rac-1 g***	**CIAP**	9 (81)	49 (13)	15	3
6		**CIP**	**2 (94)**	**-**	**-**	**-**
7	***Rac-1 h***	**CIAP**	3 (91)	75 (2)	4	7
8		**CIP**	**7 (86)**	**80 (7)**	**8**	**9**

[a]Reaction on 1 mmol of racemic ester, in 2 ml of diethyl ether and 8 ml of K_2HPO_4/Na_2HPO_4 buffer solution at 37 °C for 96 h

[b]Enantiomeric excesses measured by chiral chromatography, HPLC, or chiral GC

[c]Chemical yields are calculated after extraction

[d]Conversion rate: $C = ee_S/ee_P + ee_s$; selectivity factor $E = Ln[(1\text{-}C)(1\text{-}ee_s)]/Ln[(1\text{-}C)(1 + ee_s)]$ [54, 55]

Conclusion

In the present work, for the first time, a valorization of "chicken viscera" as a domestic organic waste of high protein content was described. The efficiency of two different biocatalyst preparations were scrutinized in the hydrolysis of fatty acid esters under sustainable conditions. The obtained results have revealed the hydrolytic propriety of this waste as potential raw animal material. Both preparations were very reactive in kinetic bioresolution of 1-phenylethyl esters via hydrolysis without selectivity. The CIAP preparation also allowed the removal of fat from fresh milk, which is particularly useful and valuable in the treatment of wastewater from dairies and oil mills. The exploitation of "chicken viscera" would reduce the negative impact of this waste on the environment, health, and development.

The UN's sustainable development agenda aims to be achieved by 2030 to reducing wastes and especially, recycling, and reuse. In Algeria, the recovery rate of this industrial wastes do not exceed of 7%. Chicken viscera can be transformed into a renewable resource and constitute a potential cheap biocatalyst source.

Finally, all these results open interesting perspectives for the recovery of this waste and could constitute a new sustainable approach for the development of more integrated poultry production systems that contribute to reducing environmental damage.

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Author Contribution Melais Nedjma: conceptualization, methodology, data curation, and original draft preparation. Louisa Aribi-Zouioueche: supervision, conceptualization, methodology, validation, reviewing, and editing. Olivier RIANT: supervision. All authors approved the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this article.

Declarations

Ethical Approval This study was performed in line with the principles of the Institutional ethical committee.

Consent to Participate This is not applicable because this study does not involve human participants.

Consent for Publication This is not applicable because this study does not involve human participants.

Competing Interests The authors declare no competing interests.

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