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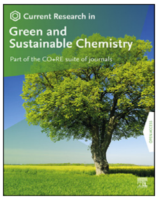

Evaluation of waste chicken feather protein hydrolysate as a bio-based binder for particleboard production

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ABSTRACT

The present study investigate the beneficiation potential of extracted keratin protein hydrolysate from chicken feather waste biomass as bio-adhesive for the production of particleboard. Chicken feathers were hydrolyzed using hybrid alkaline hydrolysis, and the obtained keratin protein fraction was used for bio-adhesive formulation. The formulated adhesive was employed for particleboard fabrication using the American National Standards Institute (A208.1) 1-L-1 grade specification. The quality of bio-adhesive and the particleboard mechanical strength performance were evaluated with Fourier transform infrared spectroscopy (FTIR), modulus of rupture (MOR), modulus of elasticity (MOE) and density. The FTIR spectra confirmed the amine, alkyl side chains and carboxylic groups of the amino acids in the unmodified keratin-based binder. The spectra revealed the covalent bonding between the azetidinium of the citric acid-based polyamide-epichlorohydrin cross-linking and the hydroxyl groups of the keratin protein hydrolysate. The fabricated particleboard's mechanical strength performance met the specification for the 1-L-1 grade of the American National Standards Institute (A208.1). The respective values obtained for modulus of rupture and modulus of elasticity of the panels made with unmodified keratin-based adhesive were 6, 5 and 1184, 34 MPa, respectively. The cellulose nanocrystals incorporation as a filler enhanced the formulated bio-adhesive static bending and bonding strength properties. Therefore, these findings demonstrate that keratin hydrolysate protein extracts from chicken feather waste could be considered as a potential feedstock for environmentally friendly wood composites bio-binder production.

1. Introduction

The manufacture of wood products like particleboard (PB), plywood (PW), oriented strand boards (OSB), medium-density fibreboards (MDF) and hardboard (HB) has continued to increase steadily[1]. This trend has gained popularity because the low quality and small-diameter trees that are not suitable for lumber manufacture can be utilized and waste biomass such as plain shavings and sawdust from sawmills. According to the FAO, worldwide wood-based composite production reached about 408 million m^3 in 2018 [2]. This production entails using wood binders as bonding agents for wood composites [3]. Currently, most of these binders are synthetic, formaldehyde-based derivatives from petroleum sources. These adhesives emit formaldehyde, which resulted in the pollution of the environment and is harmful to human health [4]. The formaldehyde is considered carcinogenic by both the Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) [5].

Furthermore, the prices of synthetic adhesives are typically dependent on the oil market with its price fluctuations. Additionally, the depletion of fossil fuel reserves is a vital concern, making accessibility of these synthetic adhesives unpredictable in the future [6]. The above problems can be alleviated by replacing the synthetic adhesives with green, environmentally sourced natural resins that can be modified to reproduce synthetic adhesives' properties and performance characteristics [7,8]. Consequently, the interest in the natural and sustainable sources of wood adhesives with similar strength properties to synthetic wood binders commonly used in the wood products industry has since been stimulated [9,10].

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Some natural materials such as tannins, lignins, carbohydrates and soy proteins have been investigated for wood adhesive potentials, and some of them are presently in use by the industry [11,12,13]. The most desirable and conventional natural-based adhesives are protein-based wood adhesives [14]. Natural proteins offer many advantages such as renewability, availability and relatively low cost that qualify them as a possible adhesive source for industrial applications [14].

There are two primary sources of protein biomass used to synthesize bio-based wood adhesives: plant and animal sources. Substantial research has been done on the bio-adhesive from plant protein sources, especially soy protein [12,15,16]. While glues derived from animal hides and bones have been used from ancient times [17], only scanty research has been carried out on animal sources of bioadhesive from slaughterhouse by-products (such as waste from poultry meat processes). Thus, Park et al. [18] investigated the adhesiveness of protein concentrate extracted from meat and bone meal (MBM) for plywood production and reported the obtained adhesive showed promising adhesion potential. The study also revealed that the modification with 0.05% glutaraldehyde improved the adhesiveness and the water-resistance of the MBM protein concentrate adhesives [18]. Moreover, utilization of waste animal protein extracts from specified risk material as bio-adhesive for both oriented strand board and plywood was studied by Mekonnen et al. [19]. The authors reported that the formulated adhesives had desirable resin requirements that make them suitable to be used in a dry environment, and the obtained binder showed less resistance to moisture [19].

Some of the disadvantages of the proteinous adhesives identified in the literature were the low mechanical strength properties of wood composites, and are much less resistant to moisture than the synthetic resins [20]. However, Adhikari et al. [1] produced and chemically modified the protein extract with polyamidoamine-epichlorohydrin (PAE). They reported that the plywood specimens made with the protein–PAE adhesive formulations lead to binders that the shear strength at dry and after soaked in water was similar to that of conventional phenol-formaldehyde adhesive [1].

This research work explores the utilization of waste chicken feathers, one of the slaughterhouse waste by-products that are yet to be fully utilized as bio-adhesives for particleboard production. According to the USA Foreign Agricultural Service, the total domestic per capita consumption of chickens in the USA and some selected countries, including South Africa in 2019, is about 96,464 $\times$ 10^3 metric tons [21].

Chicken meat is widely eating globally as part of the principal animal protein sources. Its global consumption has resulted in significant feather waste from poultry slaughterhouses. The yearly estimate of chicken feathers generated worldwide is around fifteen billion tons [22]. The Republic of South Africa currently contributes two hundred and fifty-eight million tons to the global generation of chicken feather waste from poultry slaughterhouses [22]. The bulk of the generated chicken feather waste in many countries is disposed of in landfills or burnt, and this action creates further environmental problems such as air pollution [23]. On the other hand, research into their beneficiation has gained global attention and has necessitated searching for chicken feathers' best application. Waste chicken feathers biomass could be utilized industrially because of its biodegradability, renewability, sustainability and ready availability [24].

Presently, petroleum-based adhesives for plywoods, fibreboard, particleboards are costly; hence, new interest in cheaper, environmentally friendly and renewable materials for wood adhesives are being sought after [25,26]. One of these preferred renewable materials is waste chicken feathers due to merits like its high keratin protein percentage and hydrophobicity nature, which proffer a more excellent moisture resistance character on the end product and the intrinsic property that can defend mildew fungi. These distinctive properties and the merits above make chicken feathers a potential choice of bio-adhesive feedstock for particleboard applications that requires properties like tensile strength and elasticity [27]. Waste chicken feathers are currently not exploited for valuable products at a commercial scale [27]. There is limited research on evaluating the potential bonding properties of protein extracts from chicken feather waste, especially for wood composites [25]. carried out a preliminary study on chicken feather protein-based adhesives. The adhesive was synthesized in a mixture that contained 6% sodium hydroxide and 2% sodium bisulfite with and without phenol in the solution during hydrolysis [25]. The performance of the adhesive formulations was examined by using them to produce fibreboard. The authors reported that the adhesive comprising a portion of hydrolyzed feather protein and a double part of mole ratio 1 of phenol to 2 of formaldehyde mixed at pH 10.5 performed similarly to that of conventional phenol-formaldehyde adhesive [25,28]. Earlier, in 1946, the use of waste chicken feathers as bio-binder was patented, and the alkaline hydrolyzed chicken feathers were used for the production of plywood [29]. It was reported that the plywood samples possessed mechanical strength properties similar to that of conventional plywood used in dry environments, but it exhibits low water resistance [29]. To improve both mechanical strength and moisture resistance of protein based-adhesive polyamide-epichlorohydrin (PAE) resin was used to chemically cross-linked protein hydrolysate from soy and the specified risk material, which is proteinaceous waste biomass that shows a more significant percentage of waste from the animal slaughterhouse [1,30,15] and the results were promising.

There is a scarcity of studies in the literature on waste chicken feathers-based adhesives for particleboard production. The knowledge of waste chicken feathers-based adhesive for particleboard fabrication will help valorize waste chicken feathers from slaughterhouses. Hence, beneficiation of waste chicken feathers leading to building valuable products such as particleboard and ultimately removing chicken feathers from the environment are needed. The application of waste chicken feathers adhesive for particleboard production has thus far to be studied in the valorization of waste feather biomass.

Consequently, the present study focused on using extracted protein hydrolysate from chicken feather waste through alkaline reduction hydrolysis to synthesize bio-binder for particleboard production. The potential impact of citric acid-based polyamide–epichlorohydrin (PAE) and cellulose nanocrystals as modifiers in the synthesized bio-binder on the panels' mechanical strength properties investigated.

2. Materials and methods

The waste chicken feathers used in this study were collected from Rainbow chicken slaughterhouse, Hammarsdale, KwaZulu-Natal province, Republic of South Africa. The wood particles of *Pinus pinaster* used for the production of one layer particleboard in this study were supplied by Merensky Timber, a subsidiary of Hans Merensky Holdings, Johannesburg, Republic of South Africa.

Sodium bisulphite ($NaHSO_3$, 40%), Sodium hydroxide (NaOH), ethanol (99%), and diethylenetriamine (DETA, 99%), citric acid (CA), and epichlorohydrin (ECH, 99%) used in this research work were purchased from Sigma-Aldrich (South Africa). Cellulose nanocrystals (0.5%) in suspension were supplied by Biorefinery Industry Development Facility, Council for Scientific and Industrial Research (CSIR) South Africa. FTIR spectroscopy (Frontier Universal ATR-FTIR, by PerkinElmer) was used for the functional groups' characterization of the keratin-based binders. A Willey mill and a laboratory hot press were utilized during the particleboard production process. An Instron testing machine series IX was used to characterize the mechanical strength properties of the particleboard products.

2.1. Chicken feather hydrolysis

The thermochemical hydrolysis method was used to solubilized waste chicken feathers for keratin extraction, followed the process reported in the previous study [31]. Before hydrolysis, the feathers were chemically decontaminated and, after that, dried in the oven at the temperature of 60 °C for about 24 h. After drying, ethanol was used to remove the feathers' fat by soaking for about 24 h, taking out, washed and dried in the oven for 24 h again before reducing the size with the Willey mill machine into small particles of around 1.5 mm. Milled chicken feathers (20 g) were transferred to a steel pressure vessel, and a 100 ml alkaline solution consisting of a mixture of 1.78% NaOH and 0.5% $NaHSO_3$ was added to it. The cap tight vessel was immersed in an oil bath with the temperature set at 87 °C and a reaction time of 111 min. The hydrolysate was filtered after cooling, and about 5 ml of HCl, 2 M, was added to neutralize the solution. The filtrate was dialyzed against the water using the dialysis tubing cellulose membrane (MWCO 3500-500 Da) for three days while changing the water constantly three times per day. The hydrolysate was finally removed and dried with a freeze dryer to recover the keratin hydrolysate powder, as shown in Fig. 1. The keratin hydrolysate yield was about 70%.

2.2. Protein hydrolysate yield

The percentage keratin hydrolysate yield was determined by carrying out the following calculation using the freeze-dried weight of the keratin hydrolysate and the initial weight of the feather used according to Eq. (1).

$$\text{Keratin amount } (\%) = \frac{\text{Output weight}}{\text{Feather input weight}} \times 100 \tag{1}$$

2.3. Citric acid-based polyamide-epichlorohydrin (CA-PAE) synthesis

The citric acid-based polyamide-epichlorohydrin CA-PAE was synthesized to be used as a cross-linking agent. The synthesizing process comprises two steps, as illustrated by Gui et al. [15]; with a slight modification [15]. The first step involved preparing polyamidoamine by the poly-condensation of diethylenetriamine (DETA) and citric acid (CA). However, the PAE solution was produced by dissolving the obtained polyamidoamine in water, after which it was reacted with epichlorohydrin (ECH) in an aqueous solution. The molar ratio of 1/1/1 was used for DETA/CA/ECH, respectively. Furthermore, 31 g DETA, 57.6 g CA and 20 g of water were added to the mixture and placed on a hotplate with a magnetic stirrer, a thermometer, and a condenser attached to it. Citric acid-based polyamidoamine (CA-PADA) molten was obtained after the reaction occurred under 170 °C for 90 min. Then 100 ml of water was added to dissolve the citric acid-based polyamidoamine resin. While, at the second step, 27.8 g ECH was added and mixed at room temperature for about 2 h. After that, the Citric acid-based polyamidoamine was reacted with ECH in an aqueous solution under 70 °C for 1 h to form citric acid-based polyamide-epichlorohydrin (CA-PAE). The resultant CA-PAE solution's solid content was around 50 wt %, as determined using the freeze-drying process.

2.4. Keratin-based adhesive formulation without modification

The adhesives based on the hydrolyzed keratin powder only were formulated following the method established by Adhikari et al. [32,30]; with little modification [30]. Briefly, the hydrolysate powder was dissolved based on a dry weight basis at different concentrations to achieve the solid content of 5, 10, 15, 20, 25 and 30% in a solution containing 0.5% sodium hydroxide; this is to assess the performance of the Keratin-based adhesive system without the addition of cross-linking agent. The mixture of keratin hydrolysate and sodium hydroxide solution was placed on a magnetic hot plate and stirred for about 15 min, at 70 °C. After that, the solution was left to cool down to a temperature of 25 °C before use.

2.5. Modification of the adhesive formulation with synthesized citric acid-based PAE

The effect of the citric acid-based PAE resin (cross-linking agent) modified keratin-based adhesive on the static strength properties of the resulting particleboard samples was investigated. The binder was formulated, followed the method described by Adhikari et al. [32,30]; with a slight modification, as shown in Table 1. The keratin-adhesive was developed to achieve the solid content of 5, 10, 15, 20, 25 and 30% with

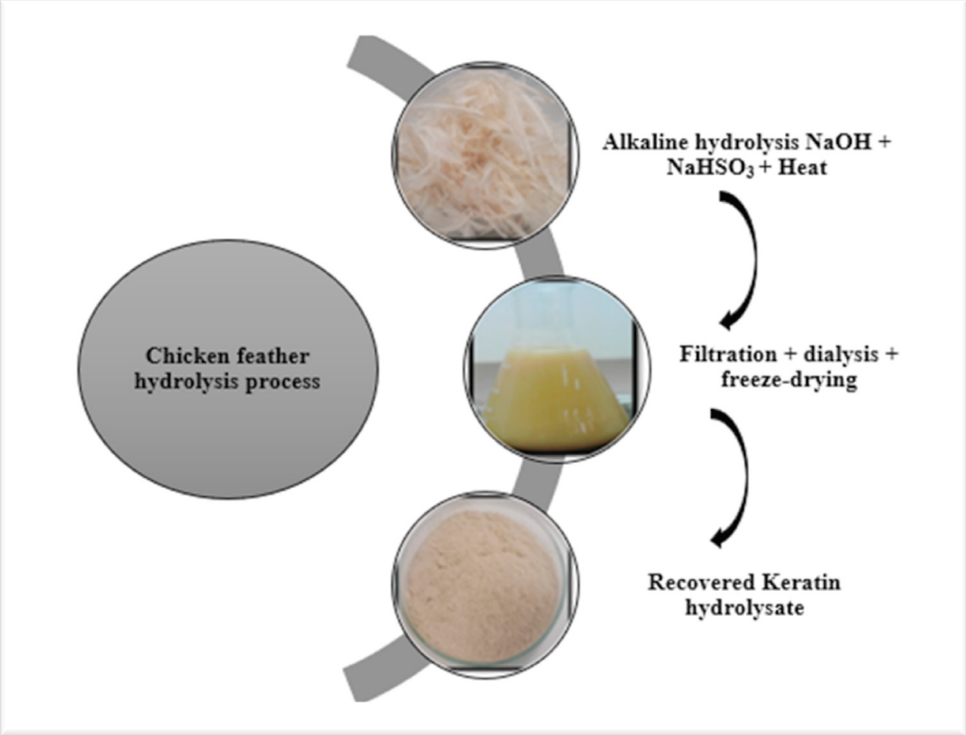

Fig. 1. Process chart showing the chicken feathers hydrolysis for keratin extraction.

Table 1

Formulations of keratin–PAE adhesive developed by varying the solid content of the formulation.

NF	PAE resin (g)	Keratin (g)	TAS (g)	DS PAE resin (g)	TS (g)	SC (%)
1	8	5	180	4	9	5
2	8	10	140	4	14	10
3	8	15	126.7	4	19	15
4	8	20	120	4	24	20
5	8	25	116	4	29	25
6	8	30	113	4	34	30

NF=Number of formulation, PAE = polyamide–epichlorohydrin, TAS = Total amount of the solution, DS = Dry solid PAE resin, TS = Total solid, SC=Solid content.

an equal amount of the cross-linking agent added to the keratin solution. The target percentage solid content for the formulated adhesive was confirmed using the freeze-drying process. This process involved weighing about 10 g of the adhesive solution into a vial, and the resultant solid content of the binder was calculated after freeze-drying according to the Equations (2) and (3) below, respectively.

$$\begin{aligned}&Solute\ output\ weight = Weight\ of\ vial\ with\ dried\ adhesive\\&\quad - Weight\ of\ empty\ vial\end{aligned} \tag{2}$$

$$Solid\ content\ (\%) = \frac{Initial\ weight - output}{Intial\ weight}\ x\ 100 \tag{3}$$

2.6. Adhesive modification with cellulose nanocrystals and synthesized PAE resin

Keratin-based adhesive modified with cellulose nanocrystals (CNC) and PAE by varying the solid from both CNC and the PAE to have a binder with a solids content of about 20% (Table 2). The formulation was done to evaluate cellulose nanocrystals' effect in the binder on the particleboard's mechanical properties. The cellulose nanocrystals were modified through the solvent exchange method by replacing the water with acetone before incorporating it into the bio-based adhesive. The solvent exchange process involved mixing acetone (99%) to the CNC suspension gradually for about 5 times, followed by centrifugation until the water was replaced by acetone. The solid content of the keratin-based binder used in this formulation was 15% with total solid of 4.5 g.

2.7. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used to study the hydrolyzed keratin protein's nature, the citric acid-based polyamide-epichlorohydrin (CA-PAE) and the bio-based adhesive formulations. The spectra were obtained in attenuated total reflection (ATR) transmission mode over a spectral range between 3500 $cm-^{1}$ and 550 $cm-^{1}$.

Table 2

Formulations of keratin–PAE adhesive developed by varying the mixing ratio of CA-PAE and CNC.

NF	PAE(g)	CNC(g)	Keratin(g)	TAS(g)	DS PAE(g)	TS(g)	SC(%)
1	5,5	0,1	4,5	35,6	2,75	7,35	20,6
2	5	0,2	4,5	35,2	2,5	7,2	20,5
3	4,5	0,3	4,5	34,8	2,25	7,05	20,3
4	4	0,4	4,5	34,4	2	6,9	20,06
5	3,5	0,5	4,5	34	1,75	6,75	19,85
6	3	0,6	4,5	33,6	1,5	6,6	19,64

NF=Number formulation, PAE = polyamide–epichlorohydrin, CNC = cellulose nanocrystals, TAS = Total amount of the solution, DS = Dry solid PAE resin, TS = Total solid, SC=Solid content.

2.8. Preparation of particleboard

The pine wood chips were milled using a Willey mill to achieve a maximum wood particle size between 1 mm and 1.25 mm. The resultant particles were oven-dried at the temperature of 60 °C for 24 h to about 6%–7% moisture content. Each panel of 700 kg/m^3 target density was prepared; the required wood sawdust was calculated using Equation (4). The weight of the materials was calculated based on the panels' target density. The adhesive needed for bonding was added to the wood particles based on the 15% of the wood particles dried in the oven initially and then mixed by hand. After the particles have been prepared, they were laid into an even and consistent mat in a steel mould with the size 218 × 75 × 40 mm, while steel block bars of about 28 mm in thickness were placed on top of the moulds for pressing into a panel thickness of approximately 10±1 mm. After mat formation, the mat was pre-pressed before hot-pressing to reduce the board mat's height and consolidate the mat for hot-pressing. The press's temperature was regulated to 180 °C at the 200 kPa pressure and press for about 15 min. Six types of one-layer particleboard panels in triplicate were produced with different adhesive formulations. The production process is shown in Fig. 2. During the manufacturing process, the hot-press temperature, pressure and time were set manually and monitored. The only parameter varied in this study was the adhesive formulation.

$$\boldsymbol{Density}\left(\frac{\boldsymbol{g}\ ^{3}}{\boldsymbol{cm}}\right) = \frac{\boldsymbol{mass}(\boldsymbol{g})}{\boldsymbol{volume}(\boldsymbol{cm}^{3}\ )} \tag{4}$$

2.9. Particleboard characterization

The fabricated boards' mechanical strength properties were assessed to determine the suitability of the keratin-based adhesive for particleboard application and the effect of the adhesive modification on the static strength and the stability in the dimension of the experimental panels. The procedures used in these tests are based on those described in ASTM D1037 (Standard Methods for Evaluating the properties of the wood base, fiber and particle panel materials) using an Instron testing machine fitted with a 5 KN load cell, operated at a rate of 5 mm/min. The specimens were tested to failure: the modulus of rupture (MOR) and the modulus of elasticity (MOE) was determined according to the formula stated in the standard [33]. The particleboard specimens cut into the 75 mm × 50 mm dimensions were used to assess the boards' dimensional stability. This analysis's sample thickness was about 9.6±1 mm, which corresponds to the mould's design. 3 samples from each of the board test series were submerged in water vertically for 2 h. Before submersion, the weight and the thickness of the specimen were measured. After 2 h, the samples were removed, drained, and the same measurement was repeated. The specimen thickness was measured using a veneer caliper, and the thickness of the boards was calculated as the mean of three measurements. The average of the data was obtained, and the percentage thickness swelling was calculated according to Equation (5). In contrast, the percentage of the experimental samples' water absorption was derived from the weight gain after soaking in water and computed mathematically based on the samples' initial weight.

The influence of some factors on the formulated adhesives was assessed on the performance of the particleboard panels. The effects of the following were evaluated (i) total solid content of the unmodified keratin-based adhesive formulation, (ii) Keratin and CA-PAE resin cross-linking, and (iii) of varying cross-linker and CNC incorporation on the properties of the particleboard panels.

$$\boldsymbol{Gt} = \frac{\boldsymbol{t}2 - \boldsymbol{t}1}{\boldsymbol{t}1} \times 100 \tag{5}$$

Where: Gt = Percentage of thickness swelling;

t2 = thickness of the sample before immersion (mm);

t1 = thickness of the sample after immersion (mm).

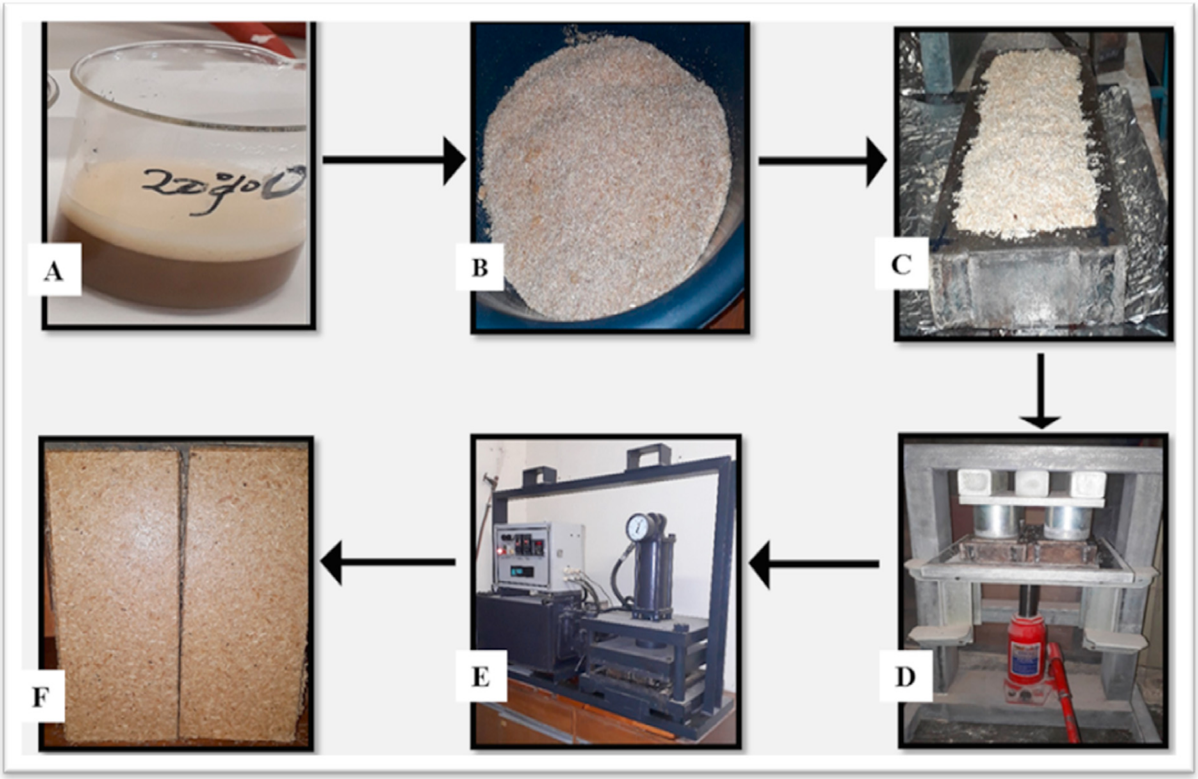

Fig. 2. Flow diagram of particleboard samples production procedure: (A) keratin-based binder; (B) wood sawdust; (C) board forming mould; (D) mat pre-press; (E) mat hot-press; (F) final board.

2.10. *Statistical evaluation*

The statistical analysis was carried out using a one-way analysis of variance (ANOVA) with Statistica software (Statsoft v13), and the mean was compared with the use of the post-hoc Fisher LSD test to determine the significance of formulation parameters on the measured properties of the particleboard panels.

3. Results and discussions

3.1. *Pre-treatment of waste chicken feathers*

The pre-treatment of waste chicken feathers was carried out to expose the polar functional groups buried within the folded protein structure for easy solubilization. These desirable active functional groups will eventually interact with the wood functional groups during the bonding process [29].

The thermochemical pre-treatment of waste chicken feathers in this study resulted in keratin hydrolyzate that afterward served as feedstock in the synthesis of keratin-based bio-adhesives. The functional group composition to illustrate the effectiveness of the pre-treatment process employed is represented in Fig. 3a. Furthermore, the resultant keratin protein showed a molecular weight of between 3 and 15 kDa using both low and medium protein molecular weight markers through the SDS-page gel electrophoresis method; this is a common molecular weight characteristic for keratin protein hydrolysate from chicken feathers [31]. The hydrolyzate recovered from the waste chicken feathers through the alkaline hydrolysis pre-treatment had sufficient protein functionality, as confirmed by the FTIR analysis (Fig. 3a), which is essential for both bio-adhesive formulation and further modification. These protein functionalities' presence leads to protein hydrolyzates' intended reaction with the citric acid-based polyamide-epichlorohydrin as the cross-linker and, eventually, the reaction adhesion by the wood particles. Additionally, the extracted keratin hydrolyzate dissolved well in a mild alkaline solution with less viscosity in resin systems. This property is desirable for the fabrication of quality wood bio-adhesives [19].

3.2. *The reaction of hydrolyzed keratin protein with CA-PAE*

In this study, the FTIR spectra (Fig. 3 a&b) show various absorption bands of protein functional groups, with different stretching vibrations of $–CH_2$, =C–H, and C–H CONH-, –OH and NH groups. The citric acid-based polyaminoamide (CA-PAE) cross-linker shows typical absorption bands of N–H, C═O, –CONH– and C–H at 3307, 1631, 1547 and 1272 $cm^{-1,}$ respectively. Functional groups such as –C-H, –CONH–, –OH and NH groups generally give absorption peaks above the wavelength of 1000 nm in the non-fingerprint region [34]. The absorption bands at 2944, 2872, and 1465 cm^{-1} correspond to the asymmetrical, symmetrical stretching vibration and bending vibration of CH_2, respectively [35]. Likewise, spectra of CA-PAE solution, cured keratin binder and keratin hydrolysate were similar. For instance, the keratin hydrolysate spectrum showed the presence of carbonyl groups (C═O stretching, absorption in the range 1650–1590 cm-1), amino group (NH stretching above 3000 cm-1 and NH bending in the range 1550–1485 cm-1) with hydroxyl groups (OH stretching above 3000 cm-1). Moreover, the FTIR spectrum for the cured keratin-based binder modified with CA-PAE resin under the temperature of 180 °C showed a similar absorption band with the keratin hydrolysate. A probable justification for the observation might be the reaction of the hydroxyl, carboxyl, and the amine group of the keratin protein hydrolyzate with the residual amine functional group of the CA-PAE cross-linker [1].

The absorption bands suggest that incorporated citric acid-based polyaminoamide has not negatively impacted the structure of the keratin-based adhesive synthesized. Instead, each component retained many of its inherent desirable properties. It is known that under very high-temperature many reactions happened in keratin-based adhesive

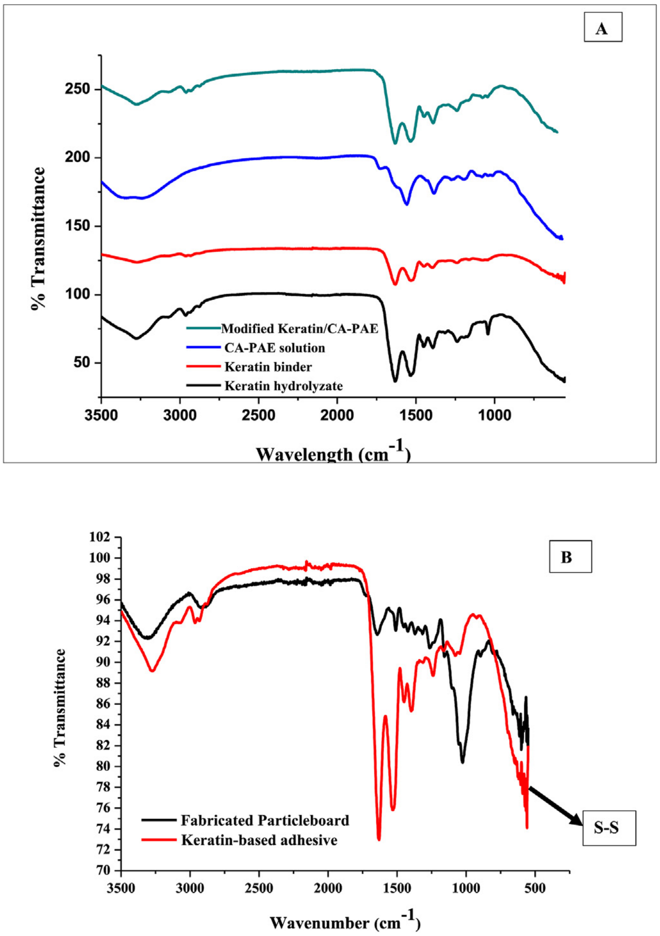

Fig. 3. (a & b): a: FTIR spectra of a keratin protein hydrolysate, cured keratin binder, citric acid-based polyamide-epichlorohydrin (CA-PAE) and keratin binder modified with CA-PAE resin. (b): Spectra showing the presence of disulphide (-S-S) in the particleboard sample.

cross-linked with CA-PAE, such as the homo-crosslinking, which occurred between the azetidium group in PAE and the remaining secondary amine within the CA-PAE resin. Besides, the interaction among the azetidinium group of CA-PAE and the active hydrogen groups of protein, like the carboxyl, hydroxyl and amino functional groups, results in co-crosslinking of the resins [36].

3.3. Performance evaluation of particleboard panels

3.3.1. Density

Table 3 and Table 4 show the panel's average density bonded with an unmodified keratin-based binder and modified keratin-based binders. The average density ranges between 690.88 kg/m^3 to 719.24 kg/m^3 for the unmodified keratin-based binder and 699.80 kg/m^3 to 727.76 kg/m^3 for the modified keratin-based binders, respectively. The target density for the experimental particleboard in this work is 700 kg/m^3. The obtained empirical density varies compared to the target density. The densities from this work is compare favourably to average actual densities reported by Amini et al. [37] that is, 0.58, 0.69 and 0.78 g/cm3 for the target densities of 0.6, 0.7 and 0.8 g/cm3 respectively. Modified starch was use as a binder for the production of particleboard from rubberwood particles in the work [37]. The variation in empirical density can be attributed to the fact that the mat-formed hot-pressed particleboard density is vertically not uniform in the thickness [38]. Particleboard density significantly influences the composites' performances and affects almost all the panel properties, including strength properties [39]. As it was stated by the American National Standards Institute (ANSI) [40]; particleboard between 0.60 and 0.8 g/cm3 is classified as medium density panels and density >0.8 g/cm3 as high-density panels [40]. Hence, the panels' density produced in this work can be classified as medium density particleboard panels. The variation in the obtained density in this work has a significant impact on the modulus of rupture (MOR) and the modulus of elasticity (MOE) of the panels.

Table 3
Panel density of the unmodified and the modified keratin-based binder.

Density (kg/m^3)		
Solid content (%)	**Keratin binder panels**	**Keratin/CA-PAE panels**
5	699,50	699,80
10	698,24	716,18
15	690,88	703,02
20	699,71	705,63
25	702,80	720,60
30	719,24	727,76

Impact of unmodified keratin-based binder solid content on static bending properties of the particleboard.

The modulus of rupture (MOR) and the modulus of elasticity (MOE) of the fabricated particleboard panels are presented in Fig. 4a and Fig. 4b. The average values for the MOR of the unmodified keratin-based binder panels range between 3,17 MPa and 6,52 MPa, respectively. Though the boards made with binder consisting of 15, 20, 25 and 30%, solid content show no significant difference. However, there is a significant difference among panels made with 5 and 10% solid content, respectively. The highest MOR was recorded with the board made with the adhesive formulation that contains 25% solid content. The literature revealed that the solid content commonly used to prepare protein-based binders for wood product fabrication are between 20 and 25% [32,41]. The bending strength of a material is determined through the modulus of rupture (MOR) and the modulus of elasticity (MOE) of the material [39]. The MOR is the highest bending stress of material in flexure or bending, while MOE is resistant to deformation or stiffness. Besides, they are used to compare one material to another and a fundamental determinant for particleboard application. The MOR results from this study are similar to what was obtained by Alawode et al. [6] with the use of modified Irvingia gabonensis and Irvingia wombolu extracts as particleboard binder [6].

The MOE result of the panels fabricated with the unmodified keratin-based binder is shown graphically in figure 4b; the average MOE values range between 644,73 MPa to 1184,34 MPa, respectively. The panels produced with 5 and 15% resin solid content showed a significant difference. In comparison, there is no significant difference with those prepared with 10 and 20% solid content, respectively. Likewise, those made with 25 and 30% solid content showed no significant differences. The panel made with the formulation that contains 5% resin solid content shows the lowest MOE, while the board made with the adhesive formulation that contains 30% solid content has the highest MOE. The difference in the static bending strength properties of the boards as a function of binder formulation could be ascribed to the extent of the binder curing, the chemical bond that is formed with the wood particles, besides the ability of a cured resin to spread [19]. However, according to ANSI [40]; the panels manufactured with 25 and 30% adhesive formulation satisfied the required specification; it could be considered under the grade 1-L-1 panel specification [40].

Table 4
Panel density of the CA-PAE and CNC modified keratin-based binder.

Mixing ratio (Panels)	1	2	3	4	5	6
Density (kg/m^3)	712,75	710,42	695,92	705,55	705,55	703,22

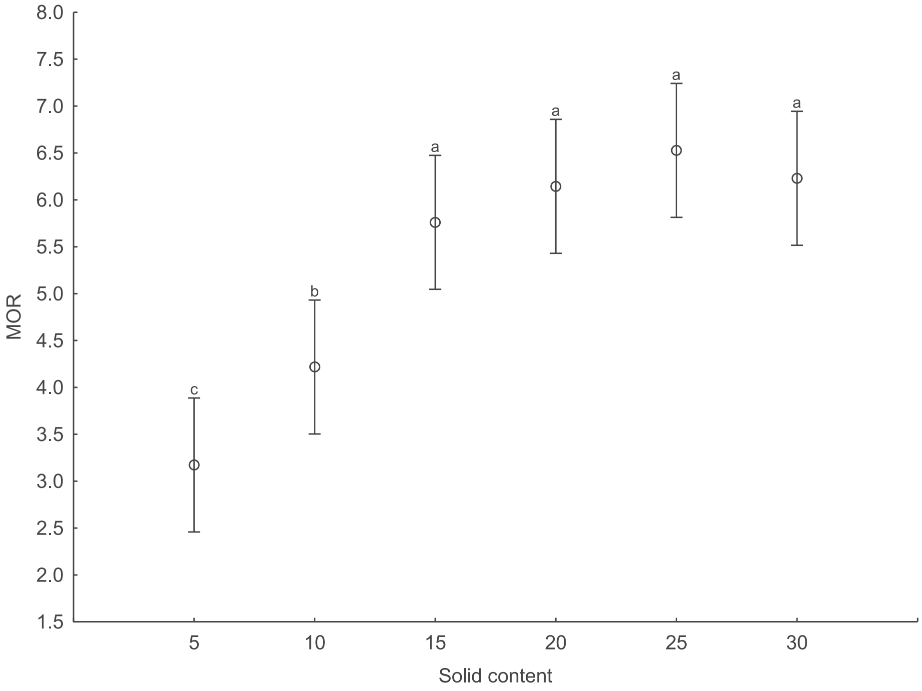

Fig. 4a. Effect of total solid content on the modulus of rupture of the particleboard samples produced with the unmodified keratin-based binder.

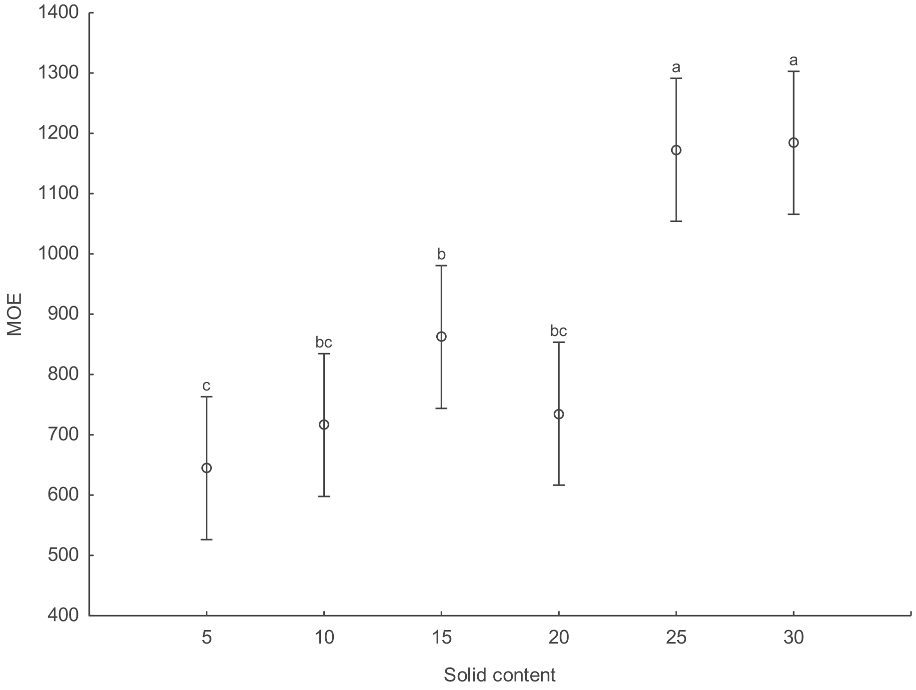

Fig. 4b. Effect of total solid content on the modulus of elasticity of the particleboard panels produced with the unmodified keratin-based binder.

3.3.2. *Effect of the cross-linking agent on the particleboard panel performance*

The MOR and MOE for citric acid incorporated polyamide-epichlorohydrin keratin-based adhesive are shown in Fig. 5a and Fig. 5b. The average MOR value ranges from 3.76 to 8.01 MPa. There are no much differences among most of the formulations evaluated, except 5% and 30% adhesive formulation, with 5% solid content adhesive panel having the lowest MOR. In comparison, the boards with adhesive of 30% solid content produced the highest MOR. Expectedly, the keratin–PAE incorporated adhesive panel showed a considerable improvement in the bending strength (MOR) compared with the unmodified keratin-based adhesive board. This improvement in the keratin–PAE adhesive panel can result from the combined influence of chemical bonding of keratin protein and CA-PAE resin molecules and the cross-linked products' reactions with the wood particles functional groups [42]. The literature revealed that chemical bonding results in the development of hard and three-D bonds of polymers linked via covalent linkages and do not allow the polymer chains from creeping during mechanical testing [1,36].

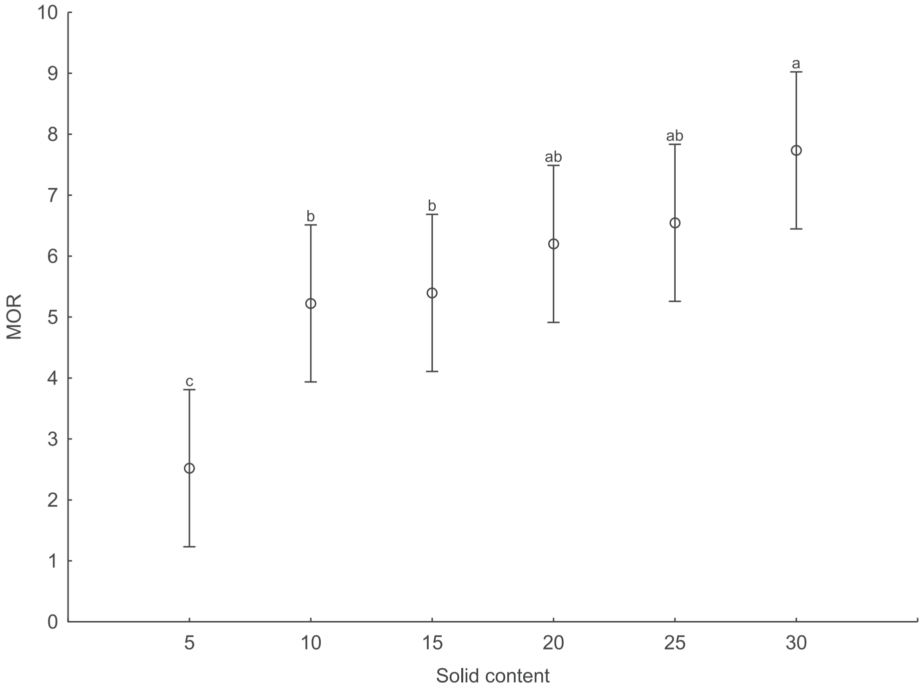

Fig. 5a. Effect of the cross-linking agent on the modulus of rupture of boards fabricated with CA-PAE cross-linked keratin-based binder.

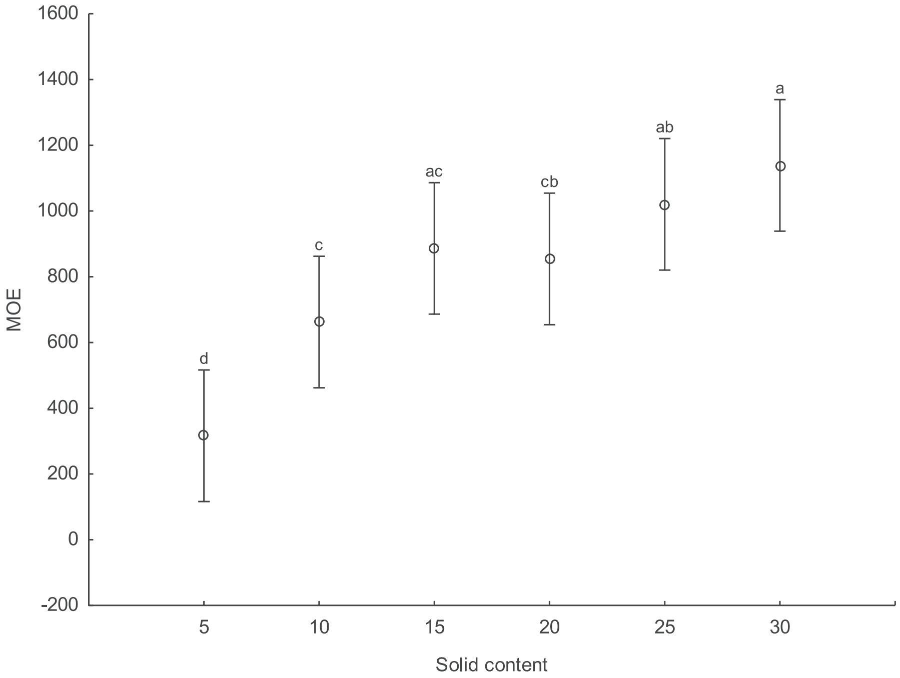

Fig. 5b. Effect of the cross-linking agent on the modulus of elasticity of boards produced with CA-PAE cross-linked keratin-based binder.

The MOE values are represented in Fig. 5b, and the MOE values range from 472, 14 to 1118,04 MPa. Most fabricated panels show no significant difference except the board fabricated with 5% solid content adhesive formulation. A decrease in the MOE performance of the keratin–PAE cross-linked adhesive panel was observed compared to the MOE of the unmodified keratin-based binder board for most of the formulations (5%, 10%, 25% and 30%) evaluated. The cause for this decrease is currently unclear; therefore, further research will be required to ascertain the reason for this observation. The average MOE obtained from this work are close to what was observed by Oliveira et al. [43]; the author reported average MOE values of 921 MPa, 1224.7 MPa and 754.1 MPa for particleboard fabricated from pines, eucalyptus and sugar bagasse, respectively [43].

3.3.3. *Effect of varying cross-linker and addition of CNC on the particleboard panel performance*

The values of the MOR and the MOE data are presented in Fig. 6a and Fig. 6b. Except for the least mixing ratio, the obtained data on the MOR of the cross-linker-CNC incorporated particleboard panel showed there are no substantial differences in the MOR values for most of the adhesive formulations implemented. The values range from 5.10 to 6.76 Mpa. The highest MOR of 6.76 Mpa was obtained in mixing ratio one formulation, which has 2.75 g and 0.1 g of CA-PAE and CNC, respectively. The result obtained from this formulation is advantageous because adhesive with low solid content has economical benefits over the binder with high solid content [44].

Furthermore, the literature reveals that, binders with very high solid content are highly viscous, leading to weak interaction of chemical and functional components and, consequently, reduction in bond strength due to the lack of effective mechanical interlocking [30,45]. Besides, previous studies show that cellulose nanocrystals' addition to wood adhesives could contribute significantly to their bond performance, thereby improving the panels' strength properties [46,47]. In line with the present study, the incorporation of cellulose nanocrystals (CNC) to particleboard adhesives had dual advantages; cellulose nanocrystals at the lowest concentration resulted in the highest modulus of rupture (MOR).

The MOE values of the cross-linker-CNC incorporated particleboard panel performance (Fig. 6b) ranges from 790.01–1232.76 MPa. The panel with mixing ratio one shows the highest MOE of 1232.76 MPa. The cross-linker-CNC incorporated particleboard panel adhesive formulations showed an improvement in particleboard strength properties compared to other fabricated panels: the unmodified keratin-based adhesive formulation and the CA-PAE cross-linker without the addition of CNC. This improvement in cross-linker-CNC incorporated particleboard panel strength might be attributed to the fact that the addition of CNC worked as a filler in the adhesive formulation to improved the strain to failure and the toughness of the adhesive and consequently the performance of the adhesive, which contributed to the mechanical strength of the fabricated particleboard panel [48].

3.4. *Thickness swelling of the particleboard panels*

Fig. 7a and Fig. 7b show the graphical representation of percentage thickness swelling of the particleboard panel obtained from the unmodified keratin-based binder, the CA-PAE cross-linked-keratin binder and the CA-PAE-CNC cross-linked keratin binder. The percentage of thickness swelling obtained after 2 h of submersion for all the particleboard panels were very high. Thus, it could not meet the ANSI minimum requirement [40]. Therefore, the present study's fabricated particleboard will be suitable as core material for doors, indoor and dry condition applications [49]. Particleboard is hygroscopic and not dimensionally stable because it is made out of wood particles; therefore, it has hygroscopic properties like wood when exposed to water vapour or liquid water [50]. Although the thickness swelling observed in the present study might be highly corresponded to the moisture-resistance of the adhesives formulated. The poor performance in water resistance could be attributed to the non-synergetic interaction of the hydrolyzed keratin proteins' active functional groups with water molecules [51]. This reaction is because the hydrogen chemical bonding gives excellent static strength performance when in a dry condition; however, the chemical bonds formed among the adhesive formulated and wood particle substrate were ruptured because of their interaction with the molecule of water [52]. The presence of partial protein in CA-PAE cross-linker network chains in the formulated binder could also result in the adhesive release from the particleboard into the water. This process would create cavities that will later permit further water circulation in the particleboard [19]. This reaction would eventually lead to lots of moisture absorption in the boards and higher percentage thickness swelling [19].

4. Conclusions

In this study, extracted keratin hydrolysate from waste chicken feather biomass was utilized as a raw material in wood adhesives for particleboard production. The FTIR results confirmed the covalent bonding of the citric acid-based polyamide-epichlorohydrin azetidinium functional group and the hydroxyl groups of the keratin protein to develop an effective co-crosslinking product. The formulated adhesives with 20, 25, and 30% solid content met the ANSI A208.1 requirements. Similarly, the mechanical strength performance of the fabricated particleboard using the formulated adhesives was promising. The addition of cellulose nanocrystals (CNC) in the adhesive formulation with 20% solid content enhanced the particleboard panel's strength performance. However, due to some functional groups of hydrolyzed keratin proteins' hydrophilic characteristics, the resistance to water of the adhesives

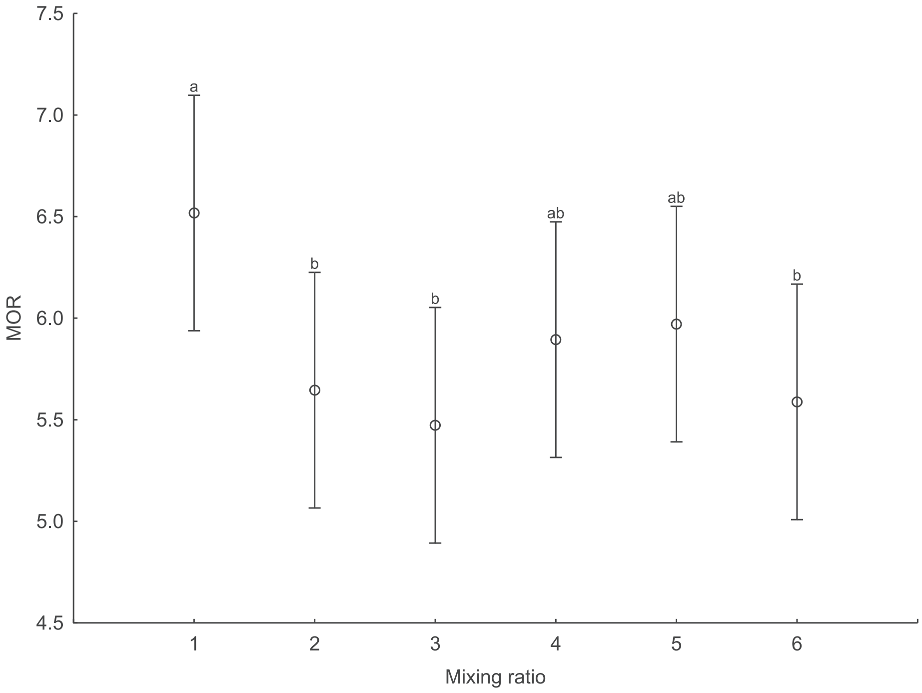

Fig. 6a. Effect of cellulose nanocrystals inclusion and mixing ratio on the modulus of rupture of panels manufacture with the CA-PAE/CNC cross-linked keratin-based binder.

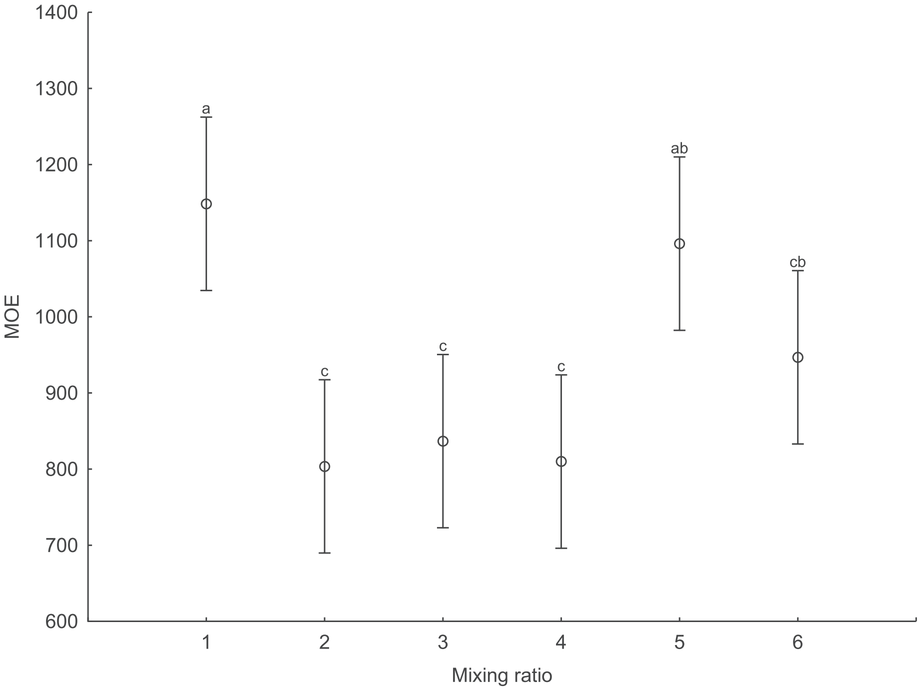

Fig. 6b. Effect of cellulose nanocrystals inclusion and mixing ratio on the modulus of elasticity of panels produced with the CA-PAE/CNC cross-linked keratin-based binder.

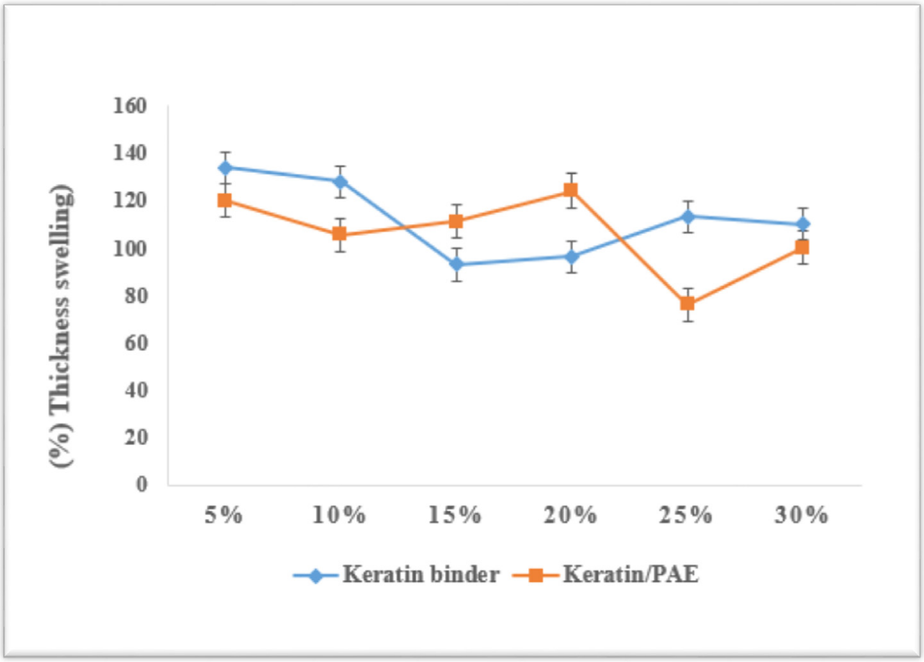

Fig. 7a. Comparison of thickness swelling of particleboards made with unmodified and cross-linked adhesive formulations.

produced did not satisfy the ANSI A208.1 specifications for structural applications. The particleboard manufacture in this research work is suggested to be utilized as a solid door core, indoor materials and dry environment applications. The valorization of the waste chicken feather to value-added products such as wood composites bio-adhesives could be expanded to other slaughterhouses proteinaceous waste and commercialization.

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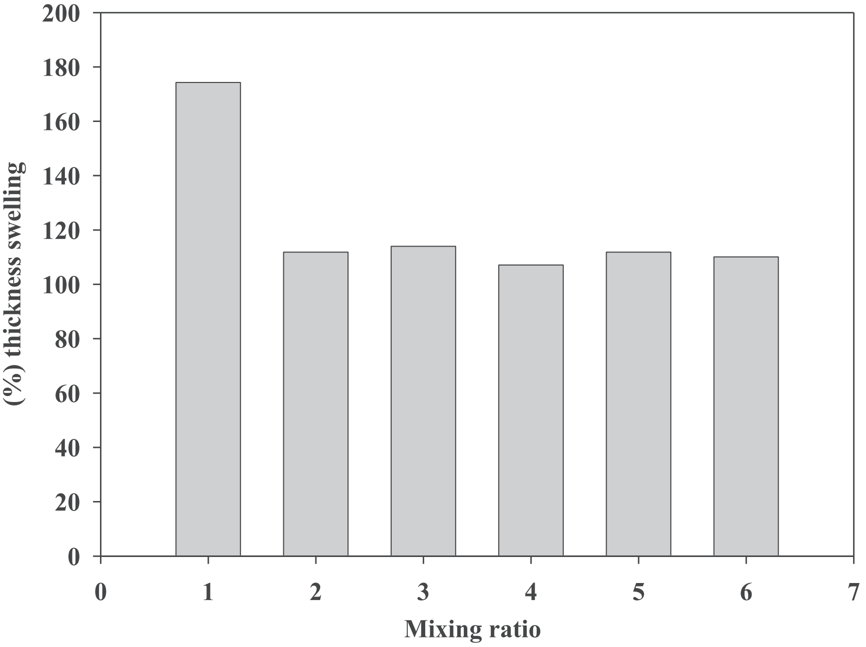

Fig. 7b. Effect of cellulose nanocrystals addition on the thickness swelling of the particleboards made with keratin-based binder modified with CA-PAE and CNC adhesive formulations.

Declaration of competing interest

The authors declare that no conflicts of interest or personal relationships could have influenced the work reported in this paper.

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