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ORIGINAL ARTICLE

Combination of ohmic heating and subcritical water to recover amino acids from poultry slaughterhouse waste at a pilot-scale: new valorization technique

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Abstract Considering the global need for waste valorization and enhancing resource efficiency, this study investigated the possibility of recovering amino acids from underutilized chicken heads and legs as poultry by-products. In this sense, a new combined technique was developed based on ohmic heating (OH) and subcritical water (SCW), i.e., OHSCW. Besides, the effects of OH at different electric field strengths (5.71, 7.14, and 8.57 V/cm) and times (15, 30, and 45 min) were compared with the control treatment (SCW equipped with conventional heating, without OH) at a temperature of 140 °C. The results showed that the come-up time using OHSCW, at electric field strengths of 7.14 and 8.57 V/cm, was less than the control method by 17 and 75%, respectively. The lowest specific energy consumption was 403.68 kJ/kg which was 59.22% less than the control method. The highest energy efficiency was 93.88% at the electric field strength of 8.57 V/cm which was superior to that of the control treatment, i.e., 47.13%. The amounts of total amino acids recovered by OHSCW, at an electric field strength of 8.57 V/cm, were higher than the control method by 70.48%. OHSCW at an electric field strength of 5.71 V/cm yielded the maximum recovery efficiency of amino acids (79.40%) while recovery efficiency in control treatment was 15.48%. Besides, the results of Amino acid Analyzer (AAA) showed that the recovered amino acids include asparagine, serine, glutamine, glycine, threonine, histidine, cysteine, alanine, aspartic, tryptophan, arginine, tyrosine, valine, methionine, isoleucine, leucine, and phenylalanine.

Keywords Ohmic heating · Subcritical water · Amino acids · Poultry wastes · Valorization

Introduction

The world is currently facing a major problem of lack of protein in food and nutrition, and to fill this deficiency, the attention of scientists has turned to the use of new sources to obtain amino acids, such as bone proteins, feathers, single-celled proteins, and poultry slaughterhouse waste (Ojha et al 2020). While the definition of poultry waste varies from a region to another, it mainly consists of feathers, skin, legs, intestines, and others parts. This also include underutilized chicken heads and legs in several countries. Inappropriate handling and disposal of slaughterhouse waste can negatively affect the quality of air and water and increases the spread of pathogenic microbes that threat of human health (Tolera and Alemu 2020). Researchers have proposed several approaches to deal with slaughterhouse waste including anaerobic digestion and synthesis of protein-based adhesive formulations, and manufacture of a protein concentrate from poultry waste (Lynch et al 2017; Dong et al 2019). Such approaches can also provide benefits to human health as the addition of protein concentrates to the diet is recommended by the manufacturers and dieticians, to provide valuable

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essential amino acids such as methionine, lysine. This also the case for minerals such as calcium and phosphorous (Olarotimi 2017). Both valuable amino acids and mineral are available in poultry waste at relatively large quantities.

Using base and the acid digestion to obtain amino acids, as a control method, damage many essential amino acids, including methionine and tryptophan, and increase the proportion of salts as a result of pH adjustment. Similarly, enzymatic hydrolysis suffers from the relatively high price of enzyme, is time consuming, and needs precise to temperature control (Salaeh et al 2020). Therefore, researchers have recently proposed innovative alternatives such as the use of subcritical water hydrolysis (SWH) for the production of peptides and amino acids (AAs). Hydrolyzing food was a unique method, to increase the value of the by-product and reduce waste (Korkmaz and Tokur 2021). Besides, subcritical water (SCW), which is water held in a liquid state under pressure between boiling point (100 °C, at pressure of 0.1 MPa) and critical point (374 °C at 22.1 MPa), might be also explored for such a process (Prado et al 2014). In this temperature range, the density of water decreases with increasing temperature and hydrogen bonds weaken. Subcritical water (SCW) was considered an environmentally friendly process (Zhan et al 2020), subcritical hydrolysis offers several advantages, including avoiding toxic chemicals, reaction time is short, minimal corrosion, low residue generation and by-product generation. The physical and chemical properties of water under these conditions change dramatically (Marcet et al 2016). At ambient temperatures, water shows a high ability to dissolve and extract ionic and polar compounds. However, when adjusting the temperature and pressure within the subcritical range, non-polar compounds can also be extracted by Go et al (2014).

Recovery of valuable compounds (e.g., amino acids) from food waste to create more sustainable and environmentally friendly processes can reduce the amount of waste. Moreover, protein recovery helps to increase the utilization of natural resources to provide further raw materials to the food industry (Marcet et al 2016). As for current state and development of amino acid recovery technology, microwave was used to improve hydrolysis of poultry waste for producing amino acids, and gave better hydrolysis compare with the traditional catalysis or subcritical water (Chen et al. 2015).

Ohmic heating (OH) is an innovative and environmentally friendly technology, which is based on passing an electric current through food and turns it into electrical resistance, it was heated by converting electrical energy into heat while the heat in the food is distributed volumetrically evenly and quickly and the direction of heat is from inside to outside (Gavahian and Chu 2021; Kostanovskii et al 2017). OH is and energy efficient technology that has been used in boiling, fermenting, steaming, peeling, cooking, sterilizing, extracting, distilling, drying and pasteurizing processes (Al-Hilphy et al 2020). However, there is no published report in the scientific database that explore the possibility of boosting SCW through the application of OH for poultry waste valorization. Therefore, the current study aimed to develop a new technique by combining OH and SCW to recover amino acids from poultry slaughterhouse waste and to evaluate the effects of processing parameters on amino acid recovery and energy consumption.

Materials and methods

Poultry slaughterhouse waste

Approximately 20 kg heads and legs (ratio of 50:50) of freshly slaughtered chicken (*Gallus domesticus*) with age of 35 days were collected from a poultry farm slaughterhouse at College of Agriculture, University of Basrah.

Preparation of the protein concentrated

The process of washing with water was carried out for the waste of poultry slaughterhouses, cooked at 140 °C for 50 min according to the method of Wiradimadja et al. (2014). The resulting fat layer was removed from it, chopped by an electric Meat Chopper (model PG520, Kenwood, China) with the hole diameter 4 mm, then dry the product with an oven dryer (model 09.14375, Binder company, Germany) at 60 °C for 12 h until it dries, then the resulting material was ground.

Amino acids recovery device

The device was designed and manufactured in the Food Engineering Laboratory at the College of Agriculture, University of Basrah (Fig. 1). It consists of a recovery cylinder made of heat-resistant Teflon, it has an inner diameter of 14.5 cm, a height of 33 cm, and a thickness of 2.5 cm. The cylinder from the bottom contains a manual valve, it was used to remove the remaining product, as well as for the washing process and the exit of water and other residues, it also contains two electrodes made of stainless steel type 316, long of each electrode 28, 5 wide and 0.01 cm thick. The cylinder was provided with a Teflon cover, 2.5 cm thick and 20 cm in diameter. A tube, a pressure gauge, a manual valve and a funnel are attached to the cover for the purpose of filling the cylinder with the liquid protein concentrate, the cover was provided with a hole through which a stainless steel tube entered into the cylinder, 26 cm long and 1.25 cm in diameter, through it, CO_2 gas enters the cylinder and connects with a bottle of CO_2 gas through a rubber tube dedicated to high pressures. There was another tube made of stainless steel that contains a manual valve that passes through the

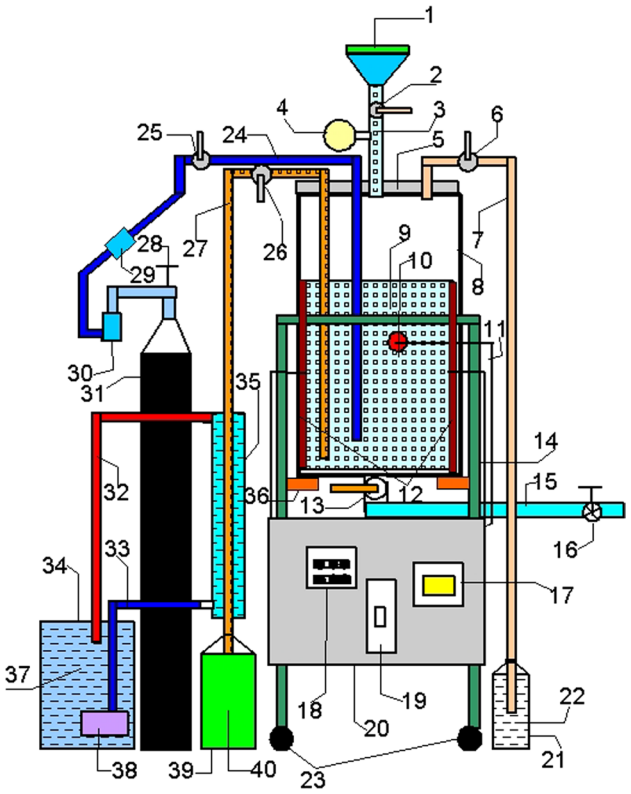

Fig. 1 OHSCW device of amino acids recovery from poultry slaughterhouse waste. 1. Funal, 2.valve, 3.pipe, 4.pressure guage, 5.tefelon cover, 6.valve, 7.pipe, 8.tefelon cylinder, 9.water and protein concentrated, 10.thermocouple, 11.wire, 12.electrodes, 13.valve, 14. body, 15.pipe, 16.valve, 17.digital temperature gauge, 18.contactor, 19.switch, 20.board, 21.bottol, 22.water, 23.tyers, 24.stainless steel pipe, 25.valve, 26.valve, 27.pipe, 28.valve, 29.flow rate meter, 30.distributor, 31.CO2 gas cylinder, 32.plastic pipe for hot water, 33.plastic pipe for cold water, 34.container, 35.heat exchanger, 36.base, 37.cold water, 38.submersable pump, 39.container to collect the product, 40.product

cover into the cylinder, 26 cm long and 1.25 cm in diameter, intended for the exit of the product, this tube was connected to a high pressure plastic tube that is connected to a glass heat exchanger. The cooling process is carried out by entering the cold water into the heat exchanger by a submersible pump in a basin containing ice water. The cooled product was then collected in a glass container. There was a tube made of stainless steel installed on the cylinder cover, 26 cm long and 1.25 cm in diameter, a manual valve was installed, and the latter is connected to a plastic tube that bears high pressure, which enters into a glass container filled with water for the purpose of expelling the gases formed during operation. The device contains a voltage changer ranging from 0–230 V and a control panel, used to operate the device by a connector and a power switch, it has a digital temperature gauge connected to it a thermocouple that measures the temperature of the product inside the retrieval cylinder.

Amino acids recovery

3 L (2 water: 1 protein concentrate) of liquid protein concentrated is put into the funnel, after opening the valve (2 and 6) and after filling, the valves are closed, CO_2 gas was pumped in and the valve is closed (6), determine voltages, operate the device for different periods of time and voltages, and measure voltages and currents for each sample using voltmeters and ammeters, to exit the product from the extraction cylinder, the valve is opened (26). Operation of the ice water pump to cool the product through the heat exchanger, collect it in a glass container and then store it at 5 °C for amino acid Analyzer (AAA) (model LC-10 AT, Koyoto Company, Japan) analysis, each sample was repeated three times. Fig. S1 illustrates the flow chart for recovery amino acids.

Control method (SCW)

Put 3 L (2 water: 1 protein concentrated) of liquid protein concentrated into a stainless steel container, closed tightly and put in it a thermocouple to measure the temperature. The container is placed on a 750 W electric heater, after the temperature reached 140 °C, the mixture was kept for different periods of time, 15, 30 and 45 min.

Electrical conductivity

The electrical conductivity (σ)is given by Eq. 1 (Içier et al 2008):

$$\sigma\left(\frac{S}{\mathrm{m}}\right) = \frac{IL}{VA} \tag{1}$$

where I is the current (A), L is the distance between electrodes (m), V is the voltage (V), and A is the section area (m^2).

Heating rate

The heating rate (H_r) is estimated according to Eq. 2:

$$H_r = \frac{T}{t} \tag{2}$$

where T is the temperature (°C), and t is the time (min).

Specific energy consumption (SEC)

SEC is calculated from Eq. 3 as follows (Raso et al 2016):

$$SEC = \frac{1}{m_w}\int_0^{\infty} V(t).I(t)dt \tag{3}$$

where *SEC* is the specific energy consumption (kJ/kg), m_w is the mass of evaporated water in the case of evaporation and drying, and mass of heated product during ohmic heating, *V* is the voltage (V), *I* is the current (A), and t is the time (s). Equation 5 is divided into 1000 to convert J to kJ.

Energy efficiency

Energy efficiency for heating mixture is calculated according to Eq. 4:

$$\eta = \frac{mC_p\left(T_f - T_i\right)}{\sum VI\Delta t} \tag{4}$$

where η is the energy efficiency (%), m is the mass of sample (kg), C_p is the specific heat capacity (kJ/kg. K), T_f is the final temperature (°C), T_i is the initial temperature (°C).

The chemical composition of the protein concentrate

Estimated percentage of ash after burning samples in an incinerator (Muffle-Furnace) Model App: 4B1554 from Galle kemp Co., England, at 600 °C for six hours until light white ash is as reported in A.O.A.C (2016). The total nitrogen in the samples was determined according to the Semi-Micro Kjeldahl method (Model 5605/B from the Swedish company Tecatorab, shown by A.O.A.C (2016) and based on the nitrogen ratio multiplied by 6.25. The percentage of crude fat was estimated based on the method used by A.O.A.C (2016) using Soxhlet Apparatus Model 456 from Tecatorab, a Swedish company, for extracting fat and using the organic solvent Petroleum ether. The percentage of crude fibers in the dried and fat extracted samples was estimated according to what was stated in A.O.A.C (2016) after treating the samples with an acidic solution and a base solution, washing, filtering, treating them with ethyl alcohol (95%), then drying them, weighing them, and burning them in a model incineration furnace, App: 4B1554 from the English company Galle kemp Co at 600 °C, then weighed and calculated the percentage of fibers. Moisture content was estimated by using oven (Binder, ED23, GmbH) at 105 °C till constant weight of concentrated protein samples. Carbohydrate was estimated according to Eq. 5.

$$\begin{aligned} &Carbohydrate = 100- \\ &(moisture\% + protein\% + ash\% + fiber\% + fat\%) \end{aligned} \tag{5}$$

Estimation of amino acids

Amino acid concentrations were estimated with the Amino acid Analyzer (model LC-10 AT, Koyoto Company, Japan). The method of digestion and extraction was as follows: 0.1 g of weight was taken from the sample (protein concentrate) and (amino acids), then acid (HCL 6 M) was added to a volume of 12 ml. Then the mixture was placed in Oven (model 561,260, Nichtdrhen, Germany) at 110 ºC for 24 h, filtered with a 0.8 µm paper filter, washed twice with distilled water, and placed in a rotary evaporator at 50 ºC, after drying, 10 ml of distilled water was added and then returned to the rotary evaporator until drying, then add acid (HCL 0.02 M) to a volume of 3.5 ml, the acidity was neutralized by adding a base. Injected into the Amino acid Analyzer device after adding OPA reagent to the sample to detect amino acids.

Recovery efficiency

The efficiency of amino acid recovery was calculated, by dividing the amino acids obtained, after recovery on the amino acids present in the sample before recovery, and as in the following equation:

$$\eta_R = \frac{AA_R}{AA_O} \times 100 \tag{6}$$

where AA_R is the number of amino acids recovered (mg/g), AA_O is the amounts of amino acids (mg/g)present in the sample before recovery, η is the recovery efficiency (%).

Statistical analysis

The complete randomized design (CRD) was used in a two-factors. The first factor was electric field strength (0, 5.71, 7.14 and 8.57 V/cm), and the second was recovery time (15, 30 and 45 min). Three replications were performed. The least significant difference (LSD) test was used to compare the means of the treatments at the 0.05 level. Each treatment was repeated three times, SPSS version 25 program was used to analyze the data.

Results and discussion

Heat performance of OHSCW

Temperature and heating rate

Figure 2a shows the temperature change of the mixture of water and protein concentrated heated with OHSCW at different values of electric field strength and control heating. The results showed that the time required to reach the temperature of 140 °C was 21 min at an electric field strength of 8.57 V/cm. The time increased with the decrease in the electric field strength. For example, the time reached 71 min at the electric field strength of 5.71 V/cm. increasing the intensity of the electric field leads to an increase in the movement

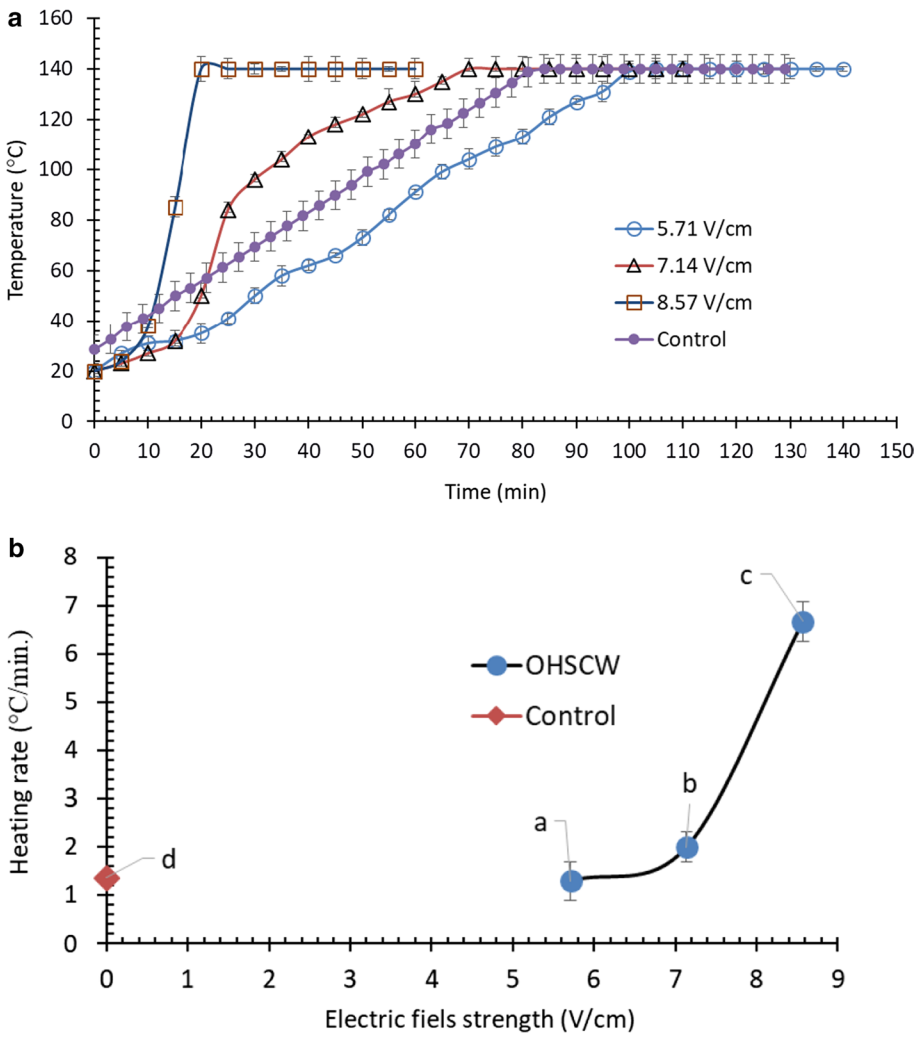

Fig. 2 Heating mixture of concentrated protein from chicken wastes and water at deferent electric field strengths. a: Temperature, and b: heating rate

of ions and the passage of a large current in the mixture, thus reducing the time required to reach the required temperature. The results also showed that the time required to reach the temperature of 140 °C is 84 min when using control treatment, which is less than the electric field strength of 5.71 V/cm due to the low supplied power (low voltage). Cho and Song (2021) stated that the OH rate and of *doenjang* was increased significantly with the increase of the electric field strength. In general, the yield of amino acids increases with increasing temperature (Zhu 2010).

The results in Fig. 3b showed that the heating rate increased significantly ($p < 0.05$) with the increase of the electric field strength, when the electric field strength is increased from 5.71 to 8.57 V/cm, the heating rate increased from 1.29- 6.66 °C/min, this increase in heating rate may be due to the passage of more current in the mixture when the electric field strength increases. Electrical energy converts into heat faster when the current passing in the mixture is increased. Gavahian et al (2019) explained the reason for the increase in the heating rate is due to the direct volumetric heating in the OH system, which can lead to a rapid increase in the temperature of the sample. These results agree with what was found by Cho et al (2016) stated that the heating rate increased from 4–45 °C/min when the electric field strength was increased from 20–60 V/cm. Al-Hilphy et al. (2020) also found that the heating rate increased from 5.33–17.5 °C/min when the electric field strength increased from 4.28–15.71 V/cm.

The results also showed that the OH at the electric field strengths 7.14 and 8.57 V/cm, it was higher than control because the heat transfer in control is slower than OH because it depends on the slow heat transfer system across hot surfaces (Gavahian et al 2019). Cho et al (2016) revealed that the heating rate in control depends on the thermal conductivity of the sample, whereas in OH, the latter depends on electrical conductivity. The results also showed that the heating rate at the electric field strength is 5.71 V/cm less than the control due to the use of a low electric voltage of 80 V.

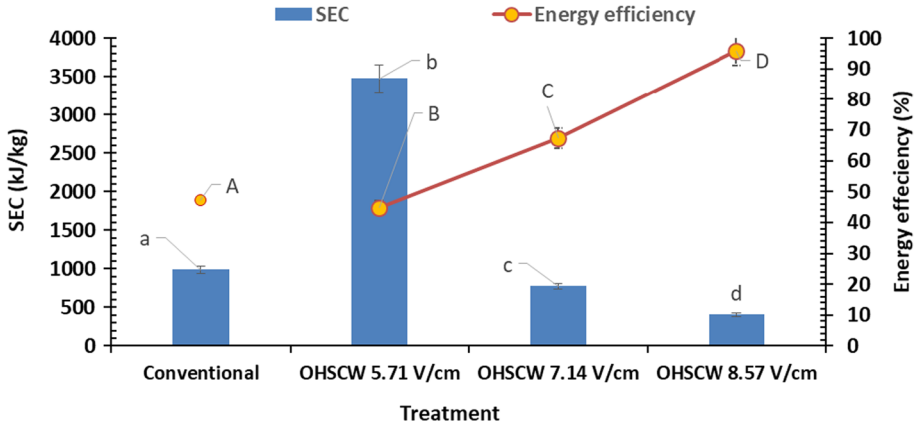

Fig. 3 SEC and energy efficiency for treatment of concentrated protein and water at deferent electric field strengths

Current, electrical conductivity

Fig. S2a shows the change of current with temperature for different values of electric field strength, the current increased with increasing temperature at all electric field strengths, when the temperature is increased from 20–140 °C, the current increased by 325%, 240%, 150% at the electric field strengths 5.71, 7.14, and 8.57 V/cm, respectively, because the movement of ions increases with increasing temperature and a large electric current pass in the mixture. It was also noticed from the results that the current passing through the mixture was higher at the electric field strength of 8.57 V/cm at all temperatures, because of the increase in the applied voltage, which leads to an increase in the supplied power and an increase in the current passing through the mixture. The current decreased slightly, the temperature is 120 °C, and the electric current passing through the mixture decreased after the temperature 59 °C, as a result of the formation of air bubbles that are insulating and also may be due to the formation of deposits on the electrodes during heating. In this study, the increase current will increase the provided power which will increase the electrical field intensity that work to tear down the protein to amino acids.

As for the electrical conductivity (Fig. S2b), the results in Figure S1b showed that the electrical conductivity increased significantly with the increase in temperature, for example, when the temperature increased from 20–140 °C, the electrical conductivity increased from 0.38–1.47 S/m at the electric field strength of 5.71 V/cm, due to the increase in the movement of ions and the decrease in viscosity with increasing temperature (Içier et al 2017). Several researchers have obtained results similar to those of the study such as Chu (2021) and Cevik (2021) who confirmed that electrical resistance decreased and electrical conductivity increases with increasing temperature. The increasing electrical conductivity leads to increase the input power which let to increase yield of amino acid due to increase temperature and reduce reaction time.

The results showed that the electrical conductivity was variable with the strength of the electric field when the temperature increased from 20–140 °C, for example, at the electric field strength of 5.71 V/cm, the electrical conductivity values ranged between 0.38–1.41 S/m, and ranged between 0.16–0.74 S/m at the electric field strength of 7.14 V/cm, and ranged from 0.38–0.79 S/m at the electric field strength. 8.57 V/cm. The reason for the decrease in the electrical conductivity with the increase in the strength of the electric field, it may be due to an increase in the current passing through the sample, which led to the formation of bubbles between the electrodes that led to a decrease in electrical conductivity, but at the beginning of heating, the current passing through is little and bubbles do not form, which led to a decrease in the conductivity with the strength of the electric field. The results also showed that the electrical conductivity was higher at the temperature less than 66 °C and at the temperature from 27–50 °C, it may be since increasing the field strength leads to an increase in the movement of fluids through the capillary property of the treated material, which is directly proportional to the electrical conductivity (Halden et al 1990) and an increase in the movement of ions. But when the temperature is increased, air bubbles are formed, as well as the formation of deposits on the surfaces of the electrodes, which leads to a decrease in electrical conductivity with an increase in the strength of the electric field.

Specific energy consumption and energy efficiency

Figure 3 shows the effect of time and electric field strength on the specific energy consumption (SEC). The results showed that the SEC decreased with the increase of the electric field strength, for example, the SECs were 3474.7, 773.1, 403.68 kJ/kg at electric field strengths of 5.72, 7.14, intensity of the electric field, these results agreed with Darvishi et al

(2015). They found that the SEC decreased with the increase of the electric field strength and showed that at lower electric field strength values, the time required for heating is greater. A number of researchers obtained similar results such as Hosainpour et al (2014). The results also showed that the SEC by OH at the electric field strengths 7.14 and 8.57 V/cm was less than the control due to the lower energy consumed and the lower heating time, the SEC when using the electric field strength of 5.71 V/cm was higher than that of control (0 V/cm) due to the increase in the heating time due to the reduced power supplied.

As for the energy efficiency (Fig. 3), it increased with the increase in the intensity of the electric field, as the energy efficiency increased from 44.89–95.88% when the electric field strength increased from 5.72–8.57 V/cm, respectively due to the decrease in the consumed power as a result of the decrease in heating time. Darvishi et al (2015) that the energy efficiency increased with the increase of the electric field strength when heating the waste mixture. The results showed that the energy efficiency of ohmic heating at the electric field strengths 7.14 and 8.57 V/cm was higher than the control due to the lower energy consumed and the lower heating time, while the energy efficiency when using the electric field strength 5.71 V/cm was lower than the control due to the increase in the heating time. Reducing specific energy consumption reduces energy requirements (saving energy and cost) for production of amino acids.

The chemical composition of the protein concentrate

Table 1 shows the chemical analysis of the locally manufactured protein concentrated from poultry slaughterhouse waste, as the percentage of protein in the produced protein concentrated was 42.50% (0.462 g/g.d.b.), as it is clear from the Table that the percentage of fat was low in the protein concentrated produced (8.65%) (0.093 g/g.d.b.). The fat, ash, moisture, and fiber reached 8.65% (0.093 g/g.d.b.), 25.95% (0.280 g/g.d.b.), 7.65% (0.083 g /g.d.b.), and 3.50% (0.038 g/g.d.b.), respectively. These results prove the success of the manufacturing process of protein concentrate from poultry slaughterhouse waste by the method used in the current study, the results were less than what was indicated by the results of Hosseinzadeh et al (2010), where the content of poultry slaughterhouse by-products powder of crude protein and crude fat was 62.12% and 25.28%, respectively, it was also lower than the results of Ahmed et al (2018), as their results showed that the percentage of protein in the protein concentrated manufactured from massacres residues was about 69.77%, while the percentage of fat was very close to our result, approximately 8.41%. The results also indicated a high percentage of ash 25.95% in the protein concentrated produced from poultry slaughterhouse residues, which is higher than what was obtained by Ahmed et al (2018), as their studies gave the ash percentage of about 18.46%, 5.26% and 10.21%, respectively.

Table 1 Chemical analysis of the locally manufactured protein

Component	Content (%) in wet weight	Content (g/g d.b) in dry weight
Protein	42.50 ± 2.55	0.462 ± 0.011
Fat	8.65 ± 1.02	0.093 ± 0.004
Ash	25.95 ± 1.23	0.280 ± 0.018
Moisture	7.65 ± 0.98	0.083* ± 0.002
Fiber	3.50 ± 0.79	0.038 ± 0.001
Carbohydrates	11.85 ± 1.43	0.128 ± 0.003

d.b.: dry basis

Amino acids recovery and recovery efficiency

Table 2 and Figs S3-S6 show the amino acids recovered (mg/g) from poultry slaughterhouse waste by OHSCW at a temperature of 140 °C. The concentration of amino acids recovered from poultry slaughterhouse waste by OHSCW at 140 °C was significantly ($P < 0.05$) affected by electric field strength and time. The results showed that the electric field intensity of 5.21 V/cm in a time of 30 min, the electric field intensity of 7.14 V/cm in a time of 45 min, the electric field intensity of 8.57 V/cm in a time of 45 min gave the highest concentration of asparagine acid, which amounted to 3.39, 3.75 and 3.26 mg/g, respectively, while the electric field strength of 5.71 V/cm in a time of 30 min and 8.57 V/cm in a time of 45 min showed the highest concentrations of serine acid (4.14 and 3.83 mg/g), respectively. Most of the amino acids outperformed at the electric field strength of 5.71 V/cm in 15 and 30 min over other electric field strength treatments at different times in all the studied amino acids. The production of amino acids was affected by temperature, as the production of amino acids increases with the increase in temperature. The reason may be attributed to an increase in the ionization constant (dissociation constant or ionic product constant) in critical water at high temperature, i.e., an increase in the concentration of hydronium and hydroxide ions, which leads to the breaking of peptide bonds in proteins or soluble amino acids that can be decomposed into carboxylic acids such as formic acid and elastic (Zhu et al 2010). Time influences the productivity of amino acids. With the extension of time, the productivity of amino acids increases to a certain extent and then decreases (Sereewatthanawut et al., 2008). Zhu et al (2010) revealed that the amino acid yield resulting from poultry slaughterhouse waste increased with the increase of the treatment time with

Table 2 Amino acids recovered (mg/g) from poultry slaughterhouse waste by OHSCW in comparison with SCW

Amino acids	OHSCW									Control (SCW) Time (min)		
	5.71 V/cm			7.14 V/cm			8.57 V/cm					
	Time (min)			Time (min)			Time (min)			Time (min)		
	15	30	45	15	30	45	15	30	45	15	30	45
Asparagine	2.79 ± 0.89^{c}	3.39 ± .09^{b}	1.18 ± 0.002^{d}	1.12 ± 0.008^{d}	1.18 ± 0.004^{d}	3.79 ± 0.005^{a}	1.18 ± 0.003^{d}	1.25 ± 0.002^{d}	3.26 ± 0.002^{b}	0.61 ± 0.002^{f}	0.95 ± 0.004^{e}	0.78 ± 0.002^{f}
Serine	1.75 ± 0.096^{d}	4.14 ± 0.02^{a}	0.57 ± 0.00^{h}	1.01 ± 0.008^{f}	1.07 ± 0.002^{g}	2.01 ± 0.003^{c}	0.57 ± 0.005^{h}	1.52 ± 0.004^{e}	3.83 ± .003^{b}	0.26 ± 0.005^{j}	0.29 ± 0.002^{j}	0.37 ± 0.004^{i}
Glutamine	3.45 ± 0.449^{b}	4.87 ± 0.130^{a}	1.43 ± 0.001^{h}	1.60 ± 0.995^{e}	1.58 ± 0.440^{f}	2.48 ± 0.003^{c}	1.43 ± 0.005^{h}	1.54 ± 0.002^{g}	2.07 ± 0.003^{d}	0.85 ± 0.005^{j}	1.00 ± 0.004^{i}	1.12 ± 0.002^{i}
Glycine	1.67 ± 0.455	2.02 ± 0.020^{b}	1.04 ± 0.004^{e}	1.22 ± 0.008^{cd}	1.32 ± 0.002^{d}	1.93 ± 0.004^{b}	1.04 ± 0.001	1.39 ± 0.001^{cd}	2.82 ± 0.003^{a}	0.33 ± 0.004^{g}	0.60 ± 0.004^{f}	0.62 ± 0.005^{f}
Threonine	2.35 ± 0.303^{b}	2.64 ± 0.201^{a}	1.47 ± 0.005^{e}	1.86 ± 0.005^{c}	0.96 ± 0.004	1.07 ± 0.003^{f}	1.47 ± 0.004^{e}	1.65 ± 0.002^{d}	2.61 ± 0.002^{a}	0.23 ± 0.005^{g}	0.37 ± 0.002^{g}	0.31 ± 0.001^{g}
Histidine	1.77 ± 0.207^{b}	2.03 ± 0.035^{a}	1.46 ± 0.002^{cd}	1.43 ± 0.004^{de}	0.58 ± 0.002^{g}	0.65 ± 0.648^{f}	1.46 ± 0.004^{cd}	1.49 ± 0.003^{c}	2.09 ± 0.001^{a}	0.12 ± 0.001	0.13 ± .002	0.14 ± 0.002
Cysteine	2.57 ± 0.153^{b}	2.95 ± 0.055^{a}	1.47 ± 0.000^{c}	1.41 ± 0.003^{c}	0.41 ± 0.003^{e}	0.48 ± 0.003^{e}	1.47 ± 0.006^{c}	1.21 ± 0.002^{d}	2.97 ± 0.003^{a}	0.24 ± 0.003^{f}	0.22 ± 0.002^{f}	0.26 ± 0.001^{f}
Alanine	2.61 ± 0.172^{b}	3.21 ± 0.104^{a}	1.76 ± 0.002^{c}	1.00 ± 0.001^{d}	1.11 ± 0.002^{d}	0.92 ± 0.003^{e}	1.76 ± 0.004^{c}	1.74 ± 0.002^{c}	2.52 ± 0.003^{b}	0.47 ± 0.004^{f}	0.59 ± 0.004^{f}	0.43 ± 0.001^{f}
Aspartic	2.83 ± 0.165^{b}	3.27 ± 0.104^{a}	1.76 ± 0.004^{d}	1.61 ± 0.003^{e}	1.70 ± 0.001^{d}	2.03 ± 0.005^{c}	1.76 ± 0.002^{c}	1.08 ± 0.002^{f}	3.14 ± 0.002^{a}	0.58 ± 0.001^{g}	0.71 ± 0.002^{g}	0.72 ± 0.004^{g}
Tryptophan	1.67 ± 0.091^{b}	1.87 ± 0.007^{b}	1.30 ± 0.004^{c}	1.28 ± 0.004^{c}	0.36 ± 0.002^{e}	0.60 ± 0.004^{d}	1.30 ± 0.001^{c}	1.29 ± 0.003^{c}	2.88 ± 0.001^{a}	0.15 ± 0.001^{f}	0.12 ± 0.001^{f}	0.13 ± 0.001^{f}
Arginine	1.72 ± 0.107^{c}	2.39 ± 0.005^{a}	1.26 ± 0.001^{d}	1.43 ± 0.004^{cd}	1.45 ± 0.004^{cd}	1.58 ± 0.004^{c}	1.26 ± 0.002^{d}	1.99 ± 0.003^{b}	2.41 ± 0.002^{a}	0.55 ± 0.001^{ef}	0.66 ± 0.005^{ef}	0.73 ± 0.005^{e}
Tyrosine	2.27 ± 0.150^{a}	2.28 ± 0.008^{a}	1.54 ± 0.001^{c}	1.02 ± 0.003^{d}	1.04 ± 0.002^{d}	1.09 ± 0.004^{d}	1.54 ± 0.002^{c}	1.57 ± 0.002^{c}	2.13 ± 0.002^{b}	0.17 ± 0.003^{e}	0.35 ± 0.005^{e}	0.33 ± 0.002^{e}
Valine	2.04 ± 0.074^{c}	3.05 ± 0.108^{a}	1.74 ± 0.0001^{d}	1.12 ± 0.003^{e}	1.14 ± 0.003^{e}	1.23 ± 0.003^{e}	1.74 ± 0.004^{d}	1.72 ± 0.002^{d}	2.52 ± 0.002^{b}	0.33 ± 0.002^{f}	0.49 ± 0.001^{f}	0.23 ± 0.002^{f}
Methionine	2.42 ± 0.598^{a}	2.48 ± 0.005^{a}	1.83 ± 0.001^{c}	1.41 ± 0.003^{d}	0.84 ± 0.003	1.50 ± 0.001^{d}	1.83 ± 0.002^{c}	1.55 ± 0.002^{d}	2.18 ± 0.002^{b}	0.17 ± 0.004	0.35 ± 0.005^{e}	0.40 ± 0.005^{e}
Isoleucine	2.44 ± 0.100^{a}	2.67 ± 0.009^{a}	1.71 ± 0.005^{b}	1.12 ± 0.003^{c}	1.16 ± 0.003^{c}	1.28 ± 0.002^{c}	1.71 ± 0.004^{b}	1.37 ± 0.003^{c}	2.47 ± 0.001^{a}	0.28 ± 0.004^{d}	0.47 ± 0.002^{d}	0.62 ± 0.005^{d}
Leucine	2.46 ± 0.055^{b}	3.00 ± 0.007^{a}	1.30 ± 0.002^{f}	1.57 ± 0.001^{e}	1.68 ± 0.003^{d}	1.57 ± 0.002^{e}	1.30 ± 0.004^{f}	1.97 ± 0.002^{c}	2.91 ± 0.002^{a}	0.75 ± 0.005^{g}	0.91 ± 0.001^{g}	0.98 ± 0.001^{g}
Phenylalanine	4.11 ± 0.004^{a}	2.37 ± 0.008^{b}	1.99 ± 0.005^{c}	1.21 ± 0.003^{ed}	1.22 ± 0.003^{ed}	1.45 ± 0.002^{d}	1.99 ± 0.003^{c}	1.58 ± 0.002^{d}	2.58 ± 0.002^{b}	0.31 ± 0.002^{g}	0.53 ± 0.001^{f}	0.60 ± 0.002^{f}
Lysine	1.78 ± 0.105^{c}	3.11 ± 0.005^{b}	1.15 ± 0.003^{f}	1.51 ± 0.002^{d}	1.56 ± 0.003^{d}	1.84 ± 0.003^{c}	1.15 ± 0.002^{f}	1.34 ± 0.002^{e}	3.53 ± 0.003^{a}	0.77 ± 0.003	0.91 ± 0.001^{g}	1.31 ± 0.002^{e}

sub-critical water and then decreased and they found that the best time for treatment is 28 min.

According the AAA results, a total numbers of 18 amino acids were identified in both OHSCW and SCW. These include Asparagine (2-amino-3-carbamoylpropanoic acid, $C_4H_8N_2O_3$), Serine ((S)-2-Amino-3-hydroxypropionic acid, $C_3H_7NO_3$), Glutamine ((S)-2,5-Diamino-5-oxopentanoic acid, $C_5H_{10}N_2O_3$), Glycine (2-Aminoethanoic acid, Glycocol, $C_8H_7N_3O_6$), Threonine ((±)-2-Amino-3-hydroxybutyric acid, $C_4H_9NO_3$), Histidine ((S)-2-Amino-3-(4-imidazolyl)propionic acid, $C_6H_9N_3O_2$), Cysteine ((S)-2-Amino-3-mercaptopropionic acid, $C_3H_7NO_2S$), Alanine (L-α-Aminopropionic acid, (S)-2-Aminopropionic acid, $C_3H_7NO_2$), Aspartic ((S)-(+)-Aminosuccinic acid, L-Aspartic acid, $C_4H_7NO_4$), Tryptophan (S)-2-Amino-3-(3-indolyl)propionic acid, $C_{11}H_{12}N_2O_2$), Arginine ((S)-2-Amino-5-guanidinopentanoic acid, $C_6H_{14}N_4O_2$), Tyrosine (L-2-Amino-3-(4-hydroxyphenyl)propanoic acid, $C_9H_{11}NO_3$), Valine (2-amino-3-methylbutanoic acid, $C_5H_{11}NO_2$), Methionine (2-amino-4-(methylthio)butanoic acid, $C_5H_{11}NO_2S$), Isoleucine (2-Amino-3-methylpentanoic acid, $C_6H_{13}NO_2$), Leucine (2-Amino-4-methylpentanoic acid, $C_6H_{13}NO_2$), and Phenylalanine ((S)-2-Amino-3-phenylpropanoic acid, $C_9H_{11}NO_2$). The retention time of the identified amino acids are reported in Table S1. Amino acids recovered (mg/g) from poultry slaughterhouse waste by OHSCW was significantly ($P < 0.05$) higher than the SCW treatments. ie. The Asparagine recovered by OHSCW at electric field strength 8.57 V/cm and time 45 min, and SCW was 3.26 and 0.78 mg/g, respectively. This is because of temperature and electrical field intensity to gather led to increase recovered amino acids like Asparagine, but using SCW only depends on the temperature. In general, the recovered amino acids by OHSCW at electrical field strength of 7.14 VV/cm and 30 min was ranged between 0.36 mg/g for Tryptophan to 4.11 mg/g for Phenylalanine at electrical field strength of 5.71 and 15 min. Moreover, the recovered amino acids by SCW at time of 30 min was ranged between 0.12 mg/g for Tryptophan to 1.12 mg/g for Glutamine at time of 45 min.

For application of the amino acid product recovered, there is an effect of biologically active peptides obtained from animal and vegetable proteins on human health. Like physiological effects, it has the effect of lowering blood pressure, lowering cholesterol, anticoagulant effects, improving mineral absorption, and immunomodulatory effects of biologically active peptides (Gilani et al 2008).

For the recovered total amino acids, it increased with increasing time (Fig. 4a) at the electric field strengths 7.14 and 8.57 V/cm, due to the increased dissociation of the protein into amino acids due to the increase in the period during which the protein concentrate is exposed to the strength of the electric field. However, at the electric field strength

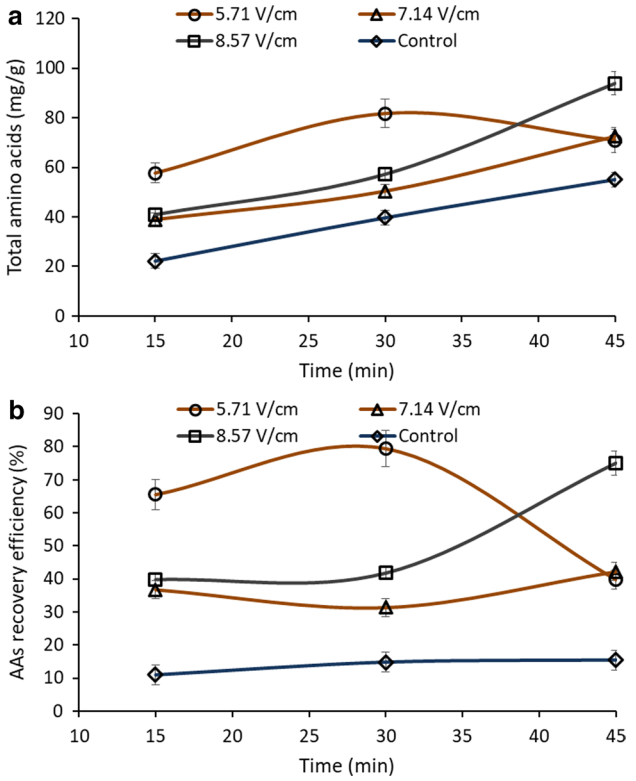

Fig. 4 Total AAs (**a**) and AAs recovery efficiency (**b**) of chicken wastes using OHSCW and control

of 5.71 V/cm, it decreased and the reason for this may be due to the increase in the dissociation of amino acids when the time increased after 30 min. The highest amount of total amino acids reached 93.92 mg/g at the electric field strength of 8.57 V/cm at the time of 45 min, due to the increase in the intensity of the electric field and time, which led to an increase in the dissociation of protein into amino acids. The minimum recovery of amino acids ranged between 22.15–55.09 mg/g when increasing the time from 15–45 min at using control treatment respectively. These results showed the superiority of Ohmic heating in cooperation with subcritical water in recovering amino acids from protein concentrates. Zhu et al (2010) stated that the amino acids recovery from poultry waste using subcritical water at 260 ℃ temperature for 1680 s was reached 0.114 mg/mg poultry waste. Amino acids yield increased with the increase of reaction time during subcritical water at a constant pressure and temperature (Zhu et al. 2010).

As for the recovery efficiency (Fig. 4b), the results showed that the highest recovery efficiency was 79.40% at the electric field strength of 5.71 V/cm at a time of 30 min, followed by 8.57 V/cm, which amounted to 75.05% at a time of 45 min, due to an increase in protein breakdown and the production of more amino acids. The results also showed

that the control treatment had a low recovery efficiency compared to the OHSCW treatments.

Conclusion

This study proposed a combination of subcritical water and ohmic heating technologies (OHSCW) to extract amino acids from poultry by-product for the first time. In comparison with control, OHSCW reduced heating time and specific energy consumption and increased the heating rate and the amounts of amino acids recovery. The amount of recovered amino acids and the efficiency of recovery increased with the increase in the recovery time. The highest and lowest concentration of amino acids using OHSCW were 0.36 mg/g for tryptophan and 4.11 mg/g for phenylalanine, respectively. A total number of 18 amino acids were identified in products of OHSCW and SCW. These include asparagine, serine, glutamine, glycine, threonine, histidine, cysteine, alanine, aspartic, tryptophan, arginine, tyrosine, valine, methionine, isoleucine, leucine, and phenylalanine. Poultry waste can be hydrolyzed to produce amino acids using OHSCW. The recovered amino acids using OHSCW was significantly ($p < 0.05$) higher than SCW. Furthermore, this method might be used in the industry to enhance resource efficiency and to help achieve the Sustainable Development Goals (SDGs) after further investigations.

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Authors' contribution ZAK: Investigation, Validation, Formal analysis, Methodology, Resources, ARA-H: Conceptualization, Writing—original draft, Software, Supervision, MHA, Methodology, Data curation. MG: Writing—review and editing, Supervision, Visualization.

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Code availability Not applicable, there is no code to be shared in this study.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

Consent to participate Not applicable/this research does not need Consent to participate.

Consent for publication Hereby, all the authors approved to transfer the copyright to the publisher of Journal of food Science and Technology if the manuscript accepted for publication.

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