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Chemical Composition and Functional Properties of Recovered Proteins from Beef Liver

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KEYWORDS

Beef liver protein, functional properties, foaming ability and stability, emulsifying ability and stability.

A B S T R A C T

This research was aimed to examine the functional properties of beef liver protein concentrates. The extraction was conducted as a function of pH and time. The pI method was applied in the purification of proteins from beef livers. Protein content of the beef liver protein concentrates was 68.69%. The functional properties of the protein concentrates were compared to those of some commercial ingredients as whey protein concentrates, and casein. Protein from beef liver exhibited better foaming properties than casein. The use of by-product proteins appears to be an interesting opportunity to obtain added value slaughterhouse by-products.

Introduction

Offal has been utilized as a one of foodstuff in Indonesia. Nollet and Toldra (2011) reported that in many countries worldwide, some by-products like the heart, liver, blood, lung, brains, kidney, tripe and spleen with high nutritional value constitute part of the diet and culinary recipes. Offal proportion from beef carcass is 16% (Ockerman and Hansen, 2000; Ockerman and Basu, 2004). In recent years, offal consumption has initially decreased, which has induced a rapid growth of the amounts of slaughterhouse by-products.

Beside for human consumption, offal is used for animal meals in pet food or through away as slaughterhouse by-products. The appearance of “mad cow” disease, also called bovine spongiform encephalopathy (BSE) has give a negative brand image of offal to consumers and has strongly restricted its use in pet food. Cholesterol content in the offal is also restricted in the consumption for health reasons. The offal is also considered as place for accumulation of pesticide, antibiotic and drug residues and

toxic chemicals and heavy metals contamination from the environment.

It is therefore necessary to find new ways to obtain added value of slaughterhouse by-products for economic and environmental reasons. For example, these by-products exhibit a high protein content, between 15 and 20% (w/w), with many essential nutrients such as amino acids, minerals, vitamins and fatty acids. The nutritional composition depends on each particular type of by-product and the animal species (Honikel, 2011). Some of them could also present interesting functional properties, but these have generally not been explored. Consequently, a new way to increase the value of slaughterhouse by-products would be to extract their proteins for use as functional ingredients in food products, for instance as water or oil holding, emulsifying, gelling or foaming agent.

Many efforts have been conducted to obtain protein from by-product sources with good functional properties. Proteins have been extracted from plant and animal (fish and livestock) sources. Plant protein usually derived from legumes. Lawal (2005) and Subagio (2006) have already extracted protein from Lablab bean with good functional properties, for example emulsifying properties, foaming property, water and oil holding capacities. Another plant protein have already been extracted from rapeseed (Der Haar *et al.*, 2014), bambarra groundnut (Lawal *et al.*, 2007), pea, chickpea and lentil seeds (Boye *et al.*, 2010; Joshi *et al.*, 2012), fenugreek (El Nasri and El Tinay, 2007), and sesame seed (Cano-Medina *et al.*, 2011; Achouri *et al.*, 2012). Fish protein have been extracted from Mackerel (Chaijan *et al.*, 2010), and anchovy (Moraes and Pinto, 2015). Lili *et al.* (2015) reported functional properties of modified egg white protein with sodium

tripolyphosphate and processed using freeze and spray drying. Yousr and Howell (2015) investigated the protein extraction from chicken egg yolk, while Zambrowicz *et al.* (2015) reported evaluation of protein from egg yolk protein by-product generated during industrial process of delipidation of yolk. Pokora *et al.* (2014) studied how enzymatic hydrolysis with a non-commercially available protease increased the use value of egg white protein preparations, generated as byproducts in the industrial process of lysizyme and cystatin isolation from egg white.

From the animal and by-product protein sources, Zouari *et al.* (2011), Pares *et al.* (2014), Alvarez *et al.* (2012), Furlan *et al.* (2010 and 2011), Salvador *et al.* (2010) and Liu *et al.* (2010) studied functional and biological properties of protein fraction from turkey liver and bovine and porcine plasma protein concentrate, respectively. Omana *et al.* (2010) attempted to extract protein from chicken dark meat using alkali method for enhanced utilization of this low value raw material. The findings suggested that the functionality of the recovered protein may provide an opportunity for greater usage of dark poultry meat. Hrynets *et al.* (2011) tried to isolate protein from mechanically separated turkey meat by a pH-shifting technique. Total protein extractability and myofibrillar protein hydrophobicity showed the same pattern, with the lowest value at pH 2.5 and the highest at pH 10.5. SDS-PAGE analysis indicated a greater concentration of myosin heavy chain dan actin in protein isolates compared to raw mechanically separated turkey meat. Selmane *et al.* (2008; 2010 and 2011) reported the extraction, production and functional properties of beef lung protein concentrates. Similarly, Conti-Silva *et al.* (2011) reported the sensory acceptability of raw and extruded bovine rumen protein in

processed meat products. With regard to the functional properties of slaughterhouse by-products, Toldra *et al.* (2016) reported and discussed the latest developments and trends in the use and valorisation of meat industry by products. Ionescu *et al.* (2008) investigated the functional properties of myofibrillar protein concentrate obtained from beef heart with transglutaminase addition. Cao and Xiong (2015) studied gelling properties of porcine myofibrillar protein with chlorogenic acid at different concentration levels (0, 6, 30 and 150 $\mu\text{mol/g}$ protein).

The aim of this research was to examine the functional properties of beef liver protein concentrates. The extraction was conducted as a function of pH and time. The pI method was applied in the purification of proteins from beef livers. Four functional properties, namely emulsifying and foaming properties, water and oil holding capacities, of the resulting concentrates were compared to those of commercial ingredients from milk (whey protein concentrates, and casein).

Materials and Methods

Raw Materials

Materials used in this research were beef liver. Beef liver protein concentrate was extracted by alkali according to Selmane *et al.* (2008). All chemicals used in this research were pro-analysis grade.

Protein Extraction

Protein extraction was conducted under mild conditions to maintain, as far as possible, their functional properties. Extraction was carried out on the beef liver using an alkali method. For each test, 200 g of by-product per 1 L water (20% w/v) were homogenized for 5 min, pH was adjusted at pH 9 with 10

M NaOH and operation time for 60 min. The suspensions were then centrifuged at 3200 rpm for 15 min and the supernatants were saved for protein concentration. Proteins were concentrated by acid precipitation (Selmane *et al.*, 2008 and 2010) at pH about 4 and adjustment was made by the use of 1 N HCl solution. This step was followed by centrifugation at 6,000 rpm 4°C for 15 min.

Proteins obtained after precipitation in the form of a paste were frozen at -20°C and dried using a microwave dryer. The protein contents of both the raw materials and the final powder were determined by using Kjeldahl method. As in the standard method, protein content was deduced from nitrogen content by multiplying the nitrogen mass fraction 6.25. Protein recovery was estimated by dividing the weight of proteins recovered in the final powder by the weight of recoverable proteins in the raw materials. Experiments were done in triplicates.

Functional properties of beef liver protein concentrate

Foaming properties

The foaming ability was measured using the method described by Selmane *et al.* (2008 and 2010). Standardized protein solutions of 2% (w/v) beef liver protein concentrates or commercial protein ingredients (whey protein concentrates and casein) were placed in test tubes, then subjected to an intense mechanical stirring for 2 min. Foaming ability was expressed by using the FA parameter expressed in percentage. Foam stability was measured using the FS parameter that corresponded to the time necessary for halving the volume of foam immediately after whipping. It is expressed in minutes. Measurements were done in triplicates.

Emulsifying properties

The emulsifying activity (EA) was determined from the turbidity of these emulsions as described by Selmane *et al.* (2008), estimated by measuring the absorbance at 500 nm using a UV–Vis spectrophotometer (Cole Palmer). EA was deduced from the following equation: $EA = 2.33 \cdot U_0$ in which U_0 is the absorbance measured just after emulsion preparation. The emulsion stability, ES, was determined by measuring the absorbance of these emulsions after 10 min. ES is expressed in minutes and is calculated by the following equation:

$$ES \text{ (min)} = 10 \cdot \frac{U_0}{U_0 - U_{10}}$$

in which U_{10} is the absorbance measured after 10 min. ES measures the rate of decrease of emulsion turbidity due to droplet coalescence and creaming, which are the key phenomena leading to emulsion destabilization. As a result, EA and ES increase when proteins favor emulsion formation and stabilization, respectively. Measurements were done in triplicates.

Water and Oil Holding Capacities

Oil holding capacity (OHC) of the protein concentrates was determined using method described by Subagio (2006) by mixing the concentrates (0.5g) with palm oil (7ml) for 1 h, then centrifuging at 2000g for 5 min.

After decantation, the sample was weighed and OHC was calculated as amount of oil trapped by the protein concentrates. Water holding capacity (WHC) of protein concentrates was determined similarly to OHC but replacing oil by water. Measurements were done in triplicates.

Results and Discussion

Beef liver analyses

The chemical composition of beef liver in term of moisture, protein, lipid, ash and carbohydrate used in this research were listed in Table 1. According to the chemical analysis (Table 1), moisture was the dominant component of the beef liver, accounting for about $70.44 \pm 3.21\%$ on a wet basis, followed by protein with $20.29 \pm 2.03\%$. The results of other researchers also suggest the high amount of protein in slaughterhouse by-products (Meshginfar *et al.*, 2014; Han *et al.*, 2014; Selmane *et al.*, 2008; Damgaard *et al.*, 2015). Table 1 also showed that beef liver constitute the most interesting by-products in terms of recoverable proteins/DM ratio with $68.69 \pm 0.66\%$. This value is similar to those of animal by-products (65.1-72.5% protein of DM basis) (Damgaard *et al.*, 2015) and goby fish protein hydrolysates (69-79% protein of DM basis) (Nasri *et al.*, 2013). This protein content of beef liver suggested that the beef liver can be a source of protein concentrate.

The amount of fat in the raw material is $3.95 \pm 1.09\%$ (based on the wet mass). Beef liver had higher fat content than values reported for turkey liver (Zouari *et al.*, 2011). This amount was drastically decreased after separation from the protein. This could due to release of fat and its sediments along with nonsoluble proteins during the high speed centrifuge (Bhaskar *et al.*, 2007). Furthermore, some of the fat was seen as a separate layer after centrifuging process on supernatant. Other researchers suggest that the amount of fat in protein concentrate is often less than 5%. The carbohydrate content of beef liver ($3.64 \pm 0.39\%$) was higher than value (1.4%) reported for turkey liver (Zouari *et al.*, 2011)

but lower than value (5.3%) obtained for beef liver (Shelf, 1975). Nevertheless, Devatkal *et al.* (2004) stated that high carbohydrate content was shown to promote growth of lactic acid bacteria on liver, resulting in a rapid spoilage.

Functional properties of the beef liver protein concentrates

Functionality has been defined as any property of a food or food ingredient, except its nutritional ones, that affects its utilization. For proteins then, there must be a large number of functions and functional properties in foods. Some of the most important ones to consider when discussing functional properties of proteins are emulsification, foam formation, gelling

property, solubility, water and oil holding capacity, viscosity, flavor binding, fiber spinning, thermal extrusion and dough formation.

The functional properties of the beef liver protein concentrates obtained in this work were measured for foaming ability (FA) and foaming stability (FS), emulsifying activity (EA) and emulsifying stability (ES), oil and water holding capacity. This functional properties of the beef liver protein concentrates were summarized in Table 2.

Table 2 showed that, except for whey proteins, beef liver proteins exhibit the highest foaming ability. Protein from beef liver exhibited better foaming properties than casein.

Table.1 Chemical composition of beef liver

Components	Amount ^c
Moisture (%)	70.44 ± 3.21
Protein (%)	20.29 ± 2.03
Lipid (%)	3.95 ± 1.09
Ash (%)	1.68 ± 0.15
Carbohydrate (%) ^b	3.64 ± 0.39
Protein/DM (%)	68.69 ± 0.66

^a calculated on wet basis

^b calculated using by difference from moisture, protein, lipid and ash.

^c Mean ± SD

Table.2 Functional properties of protein extract from beef liver compared to whey protein and casein

Protein types	Beef liver	Whey protein	Casein
FA (%)	54.6 ± 3	74.4 ± 2	48.5 ± 4
FS (min)	46 ± 2	60 ± 4	18 ± 2
EA	0.52 ± 0.01	0.44 ± 0.03	0.58 ± 0.02
ES (min)	17 ± 4	35 ± 2	16 ± 3
WHC (ml/g)	1.89 ± 0.02	2.16 ± 0.02	1.96 ± 0.01
OHC (ml/g)	6.28 ± 0.03	6.58 ± 0.04	6.42 ± 0.02

Whey proteins, as expected, exhibited the highest foam stability, which justifies their wide use as a foaming agent in the food industry. Another protein showed FS values at least 1.5 – 3 times lower. It is possible that this behavior is overshadowed by the high surface-activity of proteins. Another possibility is a stabilizing mechanism involving proteins and protein coated fat droplets, as observed in whipped cream (Selmane *et al.*, 2008).

Table 2 showed that caseins were the best emulsifying agents among the selected commercial ingredients, therefore, beef liver proteins exhibited higher EA values than whey protein concentrates. Beef liver proteins exhibited the ES values similar to casein. Such a behavior is not surprising: the low ES values of good emulsifying agents mean only that coalescence is more likely to occur rapidly when the initial turbidity A_0 is high because of higher interfacial area and higher droplet number.

WHC of the protein concentrate from beef liver was high (1.89 ± 0.02 ml/g), as shown in Table 2. This value was lower than that of whey protein concentrate and casein. The lower WHC suggested the presence of a large proportion of hydrophobic as compared to hydrophilic groups on the surface of protein molecules. Interestingly, OHC of the proteins was also lower, at 6.28 ± 0.03 ml/g.

Conclusion

The beef liver contains a high concentration of protein. The protein concentrates had good functional properties, such as foaming capacity, emulsifying capacity, water and oil holding capacity. Protein from beef liver exhibited better foaming properties than casein. The use of by-product proteins appears to be an interesting opportunity to

obtain added value slaughterhouse by-products.

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