

Effect of enzymatic pretreatment on yield and quality of animal fats obtained by low-temperature rendering*

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The growing demand for sustainable utilisation of animal by-products has increased interest in technologies that improve fat recovery while preserving the nutritional and physicochemical quality of rendered fats. The growing interest in the sustainable utilisation of animal by-products is associated with the need to reduce waste generated by the meat industry, improve resource efficiency and implement circular-economy strategies in food production. This study evaluated the effect of enzymatic pre-treatment with papain and bromelain on the efficiency of low-temperature vacuum rendering of pork and beef subcutaneous adipose tissues. Adipose tissues were incubated with

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papain (30 and 50 mg kg⁻¹) or bromelain (30 mg kg⁻¹) prior to rendering at 95±2°C under reduced pressure (200 mbar). The rendered fats were evaluated for rendering yield, fat-to-crackling ratio, physicochemical quality (acid, saponification, iodine and peroxide values), instrumental colour (CIELAB) and fatty acid composition. Enzymatic pre-treatment significantly improved rendering efficiency ($p < 0.05$). The highest rendering yield (66.85%) and fat-to-crackling ratio (2.91) were obtained for beef adipose tissue treated with 30 mg kg⁻¹ papain, whereas untreated pork adipose tissue showed the lowest values (57.98% and 1.95, respectively). Despite these improvements, acid and peroxide values remained low in all treatments, indicating limited hydrolytic and oxidative deterioration under low-temperature vacuum rendering conditions. Bromelain treatment produced the greatest changes in colour, significantly increasing yellowness (b^*), chroma and hue angle, while papain preserved the colour characteristics of beef fat close to those of the untreated control. Fatty acid composition was determined primarily by animal species rather than enzymatic treatment. Beef-derived fats contained significantly higher proportions of saturated fatty acids, stearic acid, vaccenic acid and conjugated linoleic acid (CLA), whereas pork-derived fats were characterised by higher levels of oleic acid and polyunsaturated fatty acids. Enzymatic pre-treatment did not substantially alter the nutritional fatty acid profile of the rendered fats. These findings demonstrate that proteolytic pre-treatment, particularly with papain, is an effective strategy for increasing fat recovery during low-temperature vacuum rendering while maintaining the physicochemical quality, oxidative stability and characteristic fatty acid composition of rendered animal fats. The proposed approach offers considerable potential for industrial production of high-quality edible rendered fats with improved process efficiency.

KEY WORDS: animal fats / proteolytic enzymes / papain / bromelain / low-temperature rendering / fatty acid profile

Beef fat (tallow) and pork fat (lard) serve as valuable sources of energy and unsaturated fatty acids, essential for optimal bodily function. Beef fat, particularly from grass-fed livestock, is a natural source of conjugated linoleic acid (CLA) and vaccenic acid, which are thought to possess potential anti-atherosclerotic qualities and enhance lipid and glucose metabolism, [Miciński *et al.* 2012; Yanqing Xu *et al.* 2025] and have potential antioxidant effects [Scollan *et al.* 2014]. In turn, pork lard is characterized by a high level of oleic acid, a monounsaturated fatty acid (MUFA), which has a favorable influence on the blood lipid profile [Bahelka *et al.* 2020]. Moreover, these lipids are crucial for the absorption of fat-soluble vitamins (A, D, E and K) and demonstrate significant thermal stability owing to the presence of saturated fatty acids, rendering them more stable during high-temperature heat treatment [Davis *et al.* 2022, Scollan *et al.* 2014].

Animal fats are a valuable dietary component, providing unique bioactive fatty acids and exhibiting exceptional culinary stability (pork lard smoke point of approximately 190°C, beef tallow approximately 200°C, or higher). Pork fat is distinguished by its high oleic acid content, making its fatty acid profile similar to olive oil while maintaining greater stability [Bahelka *et al.* 2020]. In the context of frying, animal fats exhibit significant advantages over vegetable oils high in polyunsaturated fatty acids (PUFAs); due to their higher proportion of saturated and monounsaturated fatty acids, they are more resistant to oxidation and thermal degradation [Kondratowicz-Pietruszka *et al.* 2019]. Studies confirm that tallow and lard generate significantly fewer toxic aldehydes and polymerization products when heated to high temperatures, allowing for safer use and longer culinary shelf life without the risk of rapid deterioration of the

fat quality [Park *et al.* 2020, Szabó *et al.* 2010, Zhuang *et al.* 2022]. Scientific research indicates a significant protective effect of saturated animal fats, such as beef tallow, on liver health, particularly in the context of protecting against alcohol-induced steatosis and liver damage. The relatively high proportions of palmitic and stearic acids characteristic of beef tallow may be relevant in the context of alcohol-associated liver injury. Recent experimental evidence indicates that long-chain saturated fatty acids, particularly palmitic and stearic acids, may attenuate ethanol-induced hepatic injury, oxidative stress, and inflammatory responses [Nie *et al.* 2022]. In contrast, diets rich in n-6 polyunsaturated fatty acids, particularly linoleic acid, have been shown in experimental models to promote lipid peroxidation, oxidative stress, inflammation, and hepatic steatosis during chronic ethanol exposure [Warner *et al.* 2017]. This mechanism is associated with the low content of linoleic acid, which limits lipid peroxidation in cell membranes and stimulates fatty acid oxidation, preventing their excessive accumulation [Ronis *et al.* 2004]. Recent reports from 2025 also suggest that moderate consumption of pork lard may contribute to reducing inflammation in adipose tissue and improving lipolytic signals, which indirectly supports hepatic metabolism [Liu *et al.* 2025]. Additionally, conjugated linoleic acid (CLA) present in beef fat has the potential to alleviate nonalcoholic fatty liver disease (NAFLD) by inhibiting triglyceride synthesis and improving insulin sensitivity [Nagao *et al.*, 2005]. Animal fats may also have a protective role against hepatotoxicity induced by certain drugs such as paracetamol, which is attributed to sparing liver glutathione stores compared to soybean oil-based diets [Hwang *et al.* 2010].

Traditional animal fats, particularly pork lard and beef tallow, have historically played an important role in Polish and European cuisine. However, the structure of dietary fat consumption in Poland changed substantially during the socioeconomic transformation that followed 1989, with a progressive shift from animal fats towards vegetable oils. More recent representative household data confirm the dominant position of vegetable oils in the Polish diet: in 2016, vegetable oils constituted the largest subgroup of added fats and provided 13.7% of total dietary fat and 29.1% of polyunsaturated fatty acid intake, whereas other animal fats accounted for only approximately 2.0% of total fat intake [Górska-Warsewicz *et al.* 2019]. This transition reflects broader changes in food availability, consumer preferences and dietary patterns accompanying economic development and transformation of the Polish food market. At the same time, dietary recommendations promoting the replacement of saturated fats with unsaturated fats have contributed to changes in the perception and selection of dietary fat sources. Recent household consumption data indicate that fats and oils remain an important component of the Polish diet. According to Statistics Poland, the average monthly household consumption of oils and fats amounted to 1.02 kg per person in 2024. Notably, animal fat consumption increased by 5.3% compared with 2023, whereas the consumption of margarine and vegetable fats decreased by 4.5%, indicating that animal fats continue to represent a relevant component of dietary fat consumption in Poland [Statistics Poland, 2025]. In the wider European context,

animal fats (from dairy, meat and added fats) remain the main source of saturated fatty acids in the diets of many nations, and a significant proportion of the EU population still exceeds the recommended standard of <10% energy from saturated fatty acids [Eilander *et al.* 2015]. However, recent years have seen a marked shift in consumer attitudes toward traditional animal fats. The long-standing dominance of low-fat dietary strategies and their limited health effects, the growing interest in authentic foods, and the renaissance of regional culinary heritage are contributing to the gradual rehabilitation of lard and tallow in consumer awareness [Frank *et al.* 2016, Szafrńska *et al.* 2021]. Market research indicates an increase in interest in fatty cuts of meat and new products combining animal and vegetable fats, demonstrating consumer openness to products based on traditional fats, especially when they are promoted in the context of taste, quality, and local tradition [Kowalska *et al.* 2026, Rosiak *et al.* 2016]. The promotion of regional and traditional meat products, including those containing lard, is part of a broader trend of appreciating Polish and European culinary heritage, gradually overcoming long-standing prejudices against animal fats [Szafrńska *et al.* 2021]. Animal fats are also an important raw material in food technology and the fat industry, serving both technological and nutritional purposes. Classic fat rendering methods rely on high-temperature processing of the raw material, which involves high energy consumption and the risk of degradation of heat-labile lipid components, including unsaturated fatty acids.[Amaryllis *et al.* 2024, Giriprasad and Goswami 2013].

An alternative to conventional methods are enzymatic processes, which utilize the ability of proteolytic enzymes to hydrolyze the protein structures surrounding fat cells. The use of enzymes can facilitate lipid release, increase process efficiency, and improve the quality of the resulting fat, while simultaneously lowering processing temperatures. The aim of this study was to evaluate the effect of enzymatic pretreatment with papain and bromelain on the efficiency of low-temperature rendering of animal fats and on the quality of the obtained lipids, in the context of potential applications in food technology and process engineering..

Material and methods

Raw material

The experimental material consisted of animal adipose tissues, including porcine subcutaneous fat and bovine subcutaneous dorsal fat. All raw materials were obtained from a single commercial meat processing plant located in eastern Poland. Porcine subcutaneous fat was collected during the deboning of chilled pig carcasses. To ensure sample consistency, two designated operators monitored the butchering process and collected the adipose tissue into dedicated containers. Bovine subcutaneous fat was collected at the end of the slaughter line immediately after completion of the slaughtering process. The collected adipose tissue was placed in plastic crates and transferred to a chilling facility. Following collection, all adipose tissue samples were vacuum-packaged, subjected to blast freezing, and stored under frozen conditions until

the technological process was initiated. The pigs used as the source of adipose tissue originated from conventional production systems. The animals were fed a balanced diet based on wheat, barley, triticale, and maize, while soybean meal and rapeseed meal served as the primary protein sources. The bovine carcasses used for adipose tissue collection originated from young cattle of dairy breeds and dairy × beef crossbreeds supplied by local producers. The animals were fed a forage-based diet consisting of hay, haylage, and maize silage, supplemented with concentrate feeds to increase dietary energy and protein levels. The concentrate ration included wheat, barley, and oat meals.

Proteolytic enzymes

Plant-derived proteolytic enzymes were used in the study. Papain (CAS No. 9001-73-4), obtained from papaya (*Carica papaya*), exhibited an activity of ≥ 6000 U mg^{-1} (USP units) and a pH of 5.0–7.0 in a 0.5% aqueous solution. Bromelain (CAS No. 37189-34-7), derived from pineapple (*Ananas comosus*), had an activity of 1200 GDU g^{-1} , was soluble in water (1% solution), and exhibited a pH of 3.0–5.0 in a 1% aqueous solution.

Enzymatic pretreatment and rendering process

Frozen adipose tissue was thawed prior to processing and transferred to an industrial meat grinder. The proteolytic enzyme preparation was uniformly applied by spraying onto the surface of the adipose tissue placed in the grinder. The tissue together with the applied enzyme was then comminuted using a grinding system equipped with two cutting knives and a grinding plate with 20-mm openings. Passage through the grinding system simultaneously reduced the particle size and facilitated uniform distribution of the enzyme preparation throughout the adipose tissue. The resulting material was subsequently transferred to a thermostatically controlled vessel for enzymatic incubation.

Following incubation, the pretreated adipose tissue was transferred to an autoclave, where the rendering process was performed according to the experimental design (Tab. 1). The procedure was conducted at a reduced pressure of 200 mBar and a temperature of $95 \pm 2^\circ\text{C}$.

Table 1. Enzyme doses and parameters for rendering beef and pork subcutaneous fat (n=6)

Group	Adipose tissue	Enzyme dose (mg/kg)	Exposure time (min)	Exposure temperature (°C)	Rendering temperature (°C)	Rendering time (min)
K1	porcine subcutaneous	0	60	65	95	90
K2	bovine subcutaneous	0	60	65	95	90
P1	porcine subcutaneous	30	60	65	95	90
P2	porcine subcutaneous	50	60	65	95	90
P3	bovine subcutaneous	30	60	65	95	90
B1	porcine subcutaneous	30	60	65	95	90
B2	bovine subcutaneous	30	60	65	95	90

P1-3 – Papain; B1-2 – Bromelain.

Determination of rendering yield

Rendering yield was determined based on the mass of fat recovered after the rendering process relative to the initial mass of the raw adipose tissue. In addition, the ratio of rendered fat to cracklings was calculated to evaluate the efficiency of fat recovery.

Acid value determination

The acid value (AV) was measured to evaluate the rancidity of the lard and beef tallow samples. The AV refers to the quantity of potassium hydroxide required to neutralize unbound fatty acids in oils or residual fats. In summary, 2 grams of lard or beef tallow was diluted with a solvent composed of 96% ethanol and diethyl ether in a 3:1 volumetric ratio. Subsequently, several drops of phenolphthalein indicator were introduced, and the solution was titrated with a sodium hydroxide titrant (0.1 M) until a pink hue manifested and persisted for no less than 30 seconds. For every experimental variety, the acid value was assessed in triplicate and computed using the subsequent formula (1):

$$AV = (5.611 \times (a-b))/m$$

in which location:

- a – volume of 0.1 M sodium hydroxide solution used for the titration of the sample (cm³);
- b – the quantity of 0.1 M sodium hydroxide solution used for the blank evaluation (cm³);
- m – sample mass (g).

Iodine value determination

The iodine value (IoV) was assessed to gauge the degree of unsaturation in the analyzed lard/beef tallow specimens. Consequently, 0.1 g of lipid was introduced into a 50 ml twist-off test tube, followed by the addition of 2.5 ml of chloroform and 3.75 ml of Hanus reagent. The concoction was agitated forcefully and positioned in a dim area for half an hour. Subsequently, 3.75 ml of 10% KI solution, 12.5 ml of distilled water, and few drops of starch solution were included. Subsequently, the combination underwent titration with a 0.1 M sodium thiosulfate solution. For every experimental variety, the iodine value was assessed in triplicate and computed using the subsequent formula:

$$IoV = (1.269 \times (a-b))/m$$

where:

- IV – iodine value (g I₂ × 100 g⁻¹ fat);
- a – volume of 0.1 M sodium thiosulfate solution required for titration of the blank (cm³);
- b – volume of 0.1 M sodium thiosulfate solution required for titration of the fat sample (cm³);
- m – sample mass (g).

Peroxide value determination

The peroxide value was determined to assess the oxidative status of the analyzed lard and beef tallow samples. Approximately 0.7 g of fat was weighed into a screw-cap test tube and dissolved in 3.5 mL of chloroform. Subsequently, 5.25 mL of glacial acetic acid and 0.7 mL of saturated potassium iodide (KI) solution were added. The reaction mixture was incubated in the dark for 5 min, after which several drops of starch indicator were introduced, and the liberated iodine was immediately titrated with 0.002 M sodium thiosulfate solution. Each fat sample variant was analyzed in triplicate, and the peroxide value was calculated using the following equation:

$$PV = ((V_s - V_o) \times C) / m \times 1000$$

where:

PV – peroxide value (meq O₂ × kg⁻¹ fat);

V_s – volume of 0.002 M sodium thiosulfate solution required for titration of the fat sample (cm³);

V_o – volume of 0.002 M sodium thiosulfate solution required for titration of the blank sample (cm³);

C – molar concentration of the sodium thiosulfate solution (mol dm⁻³);

M – mass of the analyzed fat sample (g);

1000 – conversion factor used to express the peroxide value as milliequivalents of active oxygen per kilogram of fat.

Saponification value determination

The saponification value (SV) was assessed to quantify the overall concentration of esterified fatty acids in the examined lard and beef tallow specimens. In summary, 0.5 grams of fat sample was combined with 2.5 milliliters of distilled water, 5 milliliters of 0.5 M potassium hydroxide solution, and 25 milliliters of ethanol. The reaction mixture was subjected to heating in a water bath at 95°C for 40 minutes to guarantee thorough saponification, and then cooled to ambient temperature. A control sample was concurrently generated under the same conditions, without the incorporation of the fat sample. After cooling, the sample and control solutions were titrated with 0.5 M hydrochloric acid using phenol-phthalein as an indicator until the pink hue vanished. Each experimental variant was analyzed in three independent replicates. The saponification value was determined using the subsequent equation:

$$SV = (28.55 \times (a - b)) / m$$

where:

SV – saponification value (mg KOH per gram of fat);

a – volume of 0.5 M hydrochloric acid utilized for titration of the blank sample (cm³);

- b – volume of 0.5 M hydrochloric acid utilized for titration of the fat sample (cm³);
 m – sample mass (g).

Color measurement

Color metrics (L^* , a^* , and b^*) were evaluated in the CIE Lab* color space utilizing a CR-400 chromameter (Konica Minolta Inc., Tokyo, Japan). Measurements were conducted utilizing an 8-mm aperture, D65 light source, and a 2° standard observer. The device was adjusted using a white standard plate prior to examination. Ten measurements were conducted at various sites on the surface of the chilled (2°C ±1) rendered fat samples, with the outcomes shown as average values. Furthermore, chroma (C^*), indicative of color saturation, was computed in accordance with the equation:

$$C = \sqrt{a^2 + b^2}$$

The hue angle (H°), describing the tonal attribute of the color, was subsequently calculated based on the ratio of b^* to a^* values, as shown in equation:

$$H = \arctan b/a$$

Fatty acid content determination

The fatty acid profile was determined using a modified Folch method for lipid extraction, according to the procedure described by Onopiuk et al. [25]. The isolated lipids were transformed into fatty acid methyl esters (FAME). The resultant FAMEs were examined via gas chromatography utilizing an Agilent GC-7890 gas chromatograph (Agilent Technologies, USA) fitted with a 60 m HP-88 capillary column (0.25 mm i.d. × 0.20 µm film thickness; Agilent J&W GC Columns, USA). The chromatographic parameters were established as follows: injection volume of 1 µL; split ratio of 1:40; carrier gas utilized was helium; helium flow rate set at 50 mL min⁻¹; temperatures for injector and detector maintained at 260°C. Individual fatty acids were identified by comparing their retention periods with those of a certified fatty acid standard combination (Supelco 37 Component FAME Mix, Sigma-Aldrich, USA). The relative content of individual fatty acids was expressed as a percentage of the total identified fatty acids (% total FA). Three analytical repetitions were conducted for every sample.

Statistical analysis

Three autonomous rendering batches were generated for every experimental variant. All analytical assessments were conducted separately for each batch. Statistical evaluations were conducted utilizing STATISTICA version 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The Shapiro-Wilk test was employed to evaluate the normality of data distribution, whereas Levene's test was utilized to confirm the homogeneity of variances. Upon validating the hypotheses for parametric

analysis, the disparities among experimental variants were assessed utilizing one-way analysis of variance (ANOVA), succeeded by Tukey's honestly significant difference (HSD) post hoc examination. Statistical significance was established at $p < 0.05$. Data were presented as means $\pm$ standard deviations (SD).

Results and discussion

Fat yield from rendering animal fat tissues on an industrial scale using proteolytic enzymes in pre-treatment

The present study demonstrated that enzymatic pre-treatment with papain and bromelain significantly improved the efficiency of low-temperature vacuum rendering, as evidenced by higher rendering yields and increased fat-to-crackling ratios compared with untreated controls. These findings indicate that controlled proteolysis before rendering facilitates the release of lipids from adipose tissue without increasing rendering temperature (Tab. 2).

The improved fat recovery observed after enzymatic treatment is consistent with the known mechanism of action of plant-derived cysteine proteases. Papain and bromelain hydrolyse proteins constituting the extracellular matrix, including collagen and collagen-associated proteins, thereby weakening the connective tissue network surrounding adipocytes. Disruption of this protein matrix facilitates rupture of adipocytes during thermal treatment and enhances the release of intracellular lipids [Ketnawa *et al.* 2012]. Similar mechanisms have been described in studies evaluating the use of exogenous proteases in meat processing, where enzymatic degradation of connective tissue resulted in increased collagen solubility, reduced structural integrity of tissues and improved release of cellular components [Sullivan *et al.* 2010, Ketnawa *et al.* 2012]. Among the investigated treatments, papain produced

Table 2. Rendered animal fat yield from various adipose tissues using proteolytic enzymes (n = 6)

Group	Adipose tissue	Rendered fat (kg)	Cracklings (kg)	Water (kg)	Fat to crackling ratio	Yield (%)
K1	porcine subcutaneous	870.17 $\pm$ 3.54	446.33 $\pm$ 5.54	184.50 $\pm$ 4.37	1.95 $\pm$ 0.03 ^e	57.98 $\pm$ 0.19 ^f
K2	bovine subcutaneous	932.67 $\pm$ 2.25	417.33 $\pm$ 2.73	149.83 $\pm$ 1.94	2.23 $\pm$ 0.02 ^d	62.18 $\pm$ 0.16 ^e
P1	porcine subcutaneous	944.33 $\pm$ 2.58	369.00 $\pm$ 2.37	183.00 $\pm$ 4.24	2.56 $\pm$ 0.02 ^c	63.10 $\pm$ 0.18 ^d
P2	porcine subcutaneous	960.17 $\pm$ 3.66	352.00 $\pm$ 8.00	183.67 $\pm$ 5.65	2.73 $\pm$ 0.07 ^b	64.17 $\pm$ 0.20 ^c
P3	bovine subcutaneous	1004.67 $\pm$ 3.83	344.83 $\pm$ 5.27	153.67 $\pm$ 1.03	2.91 $\pm$ 0.05 ^a	66.85 $\pm$ 0.25 ^a
B1	porcine subcutaneous	943.17 $\pm$ 2.86	375.83 $\pm$ 8.70	183.50 $\pm$ 6.38	2.51 $\pm$ 0.06 ^c	62.77 $\pm$ 0.24 ^d
B2	bovine subcutaneous	997.00 $\pm$ 3.16	356.50 $\pm$ 3.02	149.33 $\pm$ 3.83	2.8 $\pm$ 0.03 ^b	66.33 $\pm$ 0.22 ^b

K1 – pork subcutaneous adipose tissue; K2 – beef subcutaneous adipose tissue; P1 – pork subcutaneous adipose tissue with 30mg/kg papain; P2 – pork subcutaneous adipose tissue with 50mg/kg papain; P3 – beef subcutaneous adipose tissue with 30mg/kg papain; B1 – pork subcutaneous adipose tissue with 30mg/kg bromelain; B2 – beef subcutaneous adipose tissue with 30mg/kg bromelain.

ab... Values are reported as mean $\pm$ SD; different lowercase superscript letters in the same column indicate statistically significant differences between experimental groups ($p < 0.05$).

the greatest improvement in rendering performance, particularly in beef adipose tissue. Beef adipose tissue pre-treated with 30 mg/kg papain (P3) exhibited the highest rendering yield (66.85%) and the highest fat-to-crackling ratio (2.91), both significantly higher than those obtained for bromelain-treated samples. Papain is characterised by broad substrate specificity and high proteolytic activity towards connective tissue proteins, including collagen-associated proteins and elastin. Previous studies have shown that papain generally produces more extensive degradation of connective tissue than bromelain under comparable processing conditions, resulting in greater structural disintegration and improved extraction efficiency [Arshad *et al.* 2016]. These observations are consistent with the higher rendering efficiency obtained in the present study. Although bromelain also significantly improved rendering efficiency compared with untreated controls, its effect was less pronounced than that of papain in beef adipose tissue. Bromelain exhibits high affinity towards collagen and other extracellular matrix proteins; however, differences in catalytic activity, substrate specificity and enzyme composition may influence its effectiveness depending on tissue characteristics and processing conditions [Ketnawa *et al.* 2012, Arshad *et al.* 2016]. Nevertheless, bromelain treatment substantially increased both rendering yield and fat-to-crackling ratio compared with untreated adipose tissue, confirming its usefulness as a technological aid for fat rendering.

An important finding of the present study was the strong relationship between rendering yield and the fat-to-crackling ratio. Treatments producing the highest fat-to-crackling ratios also exhibited the highest rendering yields, indicating that enzymatic hydrolysis reduced the amount of lipid retained within the cracklings. Since the initial adipose tissue mass did not differ significantly among experimental groups, these differences can be attributed primarily to more efficient lipid release from the connective tissue matrix rather than to differences in the quantity of processed raw material. Differences between pork and beef adipose tissues were also evident. Even without enzymatic treatment, beef adipose tissue exhibited significantly higher rendering yield than pork adipose tissue. Such differences are consistent with previous reports demonstrating that adipose tissues differ in collagen organisation, extracellular matrix composition and connective tissue architecture depending on animal species and anatomical location [Christensen *et al.* 2016]. These structural characteristics influence tissue susceptibility to proteolytic degradation and consequently affect the efficiency of lipid release during thermal processing.

Overall, the present results demonstrate that enzymatic pre-treatment represents an effective approach for improving fat recovery during low-temperature vacuum rendering. The observed increases in rendering yield and fat-to-crackling ratio indicate that controlled proteolysis enhances disruption of the connective tissue matrix, thereby facilitating lipid separation without the need to increase rendering temperature. Among the enzymes investigated, papain proved to be the most effective under the applied processing conditions.

Chemical quality

The physicochemical characteristics of the rendered fats indicate that both the origin of adipose tissue and enzymatic pre-treatment influenced fat quality after low-temperature vacuum rendering. Although significant differences were observed among experimental groups, all rendered fats exhibited relatively low acid and peroxide values, indicating limited hydrolytic and oxidative deterioration during processing (Tab. 3).

The acid value is widely recognised as an indicator of hydrolytic degradation and reflects the concentration of free fatty acids released from triacylglycerols. In the present study, rendered fats obtained from beef adipose tissue exhibited significantly higher acid values than those produced from pork adipose tissue, irrespective of enzymatic treatment. This observation suggests that the intrinsic characteristics of the raw material had a greater influence on free fatty acid formation than the applied proteolytic enzymes. Similar differences between animal fat sources have been reported previously and are associated with variations in lipid composition, endogenous lipase activity and tissue structure [Shahidi 2005, Gunstone *et al.* 2007]. Importantly, despite the observed differences, all acid values remained generally accepted for edible rendered fats, indicating good overall fat quality.

Proteolytic pre-treatment did not markedly increase acid value compared with the corresponding controls. This finding suggests that papain and bromelain primarily hydrolysed structural proteins of the connective tissue rather than promoting lipid hydrolysis. Since both enzymes are cysteine proteases with no intrinsic lipolytic activity, their technological effect

is expected to be limited mainly to degradation of extracellular matrix proteins, facilitating lipid release without directly accelerating triacylglycerol hydrolysis [Ketnawa *et al.* 2012].

Table 3. Acid value, saponification value, iodine value and peroxide value of various adipose tissues using proteolytic enzymes

Group	Acid value (mg KOH x g ⁻¹ (-1))	Saponification value (mg KOH x g ⁻¹ (-1) fat)	Iodine value (g I 2 100 x g ⁻¹ (-1) fat)	Peroxide value (meq O 2 [(x kg)] ⁻¹ (-1) fat)
K1	0.53 ± 0.06 ^e	188.02 ± 0.16 ^a	63.00 ± 0.34 ^a	2.79 ± 0.03 ^b
K2	1.49 ± 0.16 ^a	181.22 ± 0.07 ^b	53.97 ± 0.52 ^a	2.80 ± 0.02 ^b
P1	0.43 ± 0.06 ^d	192.19 ± 7.16 ^a	47.78 ± 4.92 ^a	3.05 ± 0.59 ^b
P2	0.51 ± 0.13 ^c	180.06 ± 2.67 ^b	31.12 ± 4.36 ^b	2.80 ± 0.03 ^b
P3	1.12 ± 0.07 ^b	199.40 ± 1.96 ^a	52.45 ± 1.14 ^a	3.69 ± 0.80 ^b
B1	0.56 ± 0.03 ^c	182.73 ± 7.27 ^b	35.67 ± 4.00 ^b	6.51 ± 0.84 ^a
B2	1.17 ± 0.03 ^b	205.64 ± 3.38 ^a	35.67 ± 4.00 ^b	4.13 ± 0.10 ^b

K1 – pork subcutaneous adipose tissue; K2 – beef subcutaneous adipose tissue; P1 – pork subcutaneous adipose tissue with 30mg/kg papain; P2 – pork subcutaneous adipose tissue with 50mg/kg papain; P3 – beef subcutaneous adipose tissue with 30mg/kg papain; B1 – pork subcutaneous adipose tissue with 30mg/kg bromelain; B2 – beef subcutaneous adipose tissue with 30mg/kg bromelain.

ab...Values are reported as mean±SD; different lowercase superscript letters in the same column indicate statistically significant differences between experimental groups (p<0.05).

The saponification value differed significantly among treatments, with the highest values observed in beef adipose tissue treated with bromelain and papain. The saponification value is inversely related to the average molecular weight of fatty acids present in triacylglycerols and therefore reflects differences in fatty acid composition rather than lipid degradation [Gunstone *et al.* 2007]. Because proteolytic enzymes do not chemically modify fatty acids, the observed differences most likely resulted from selective release of lipid fractions associated with specific adipose tissue structures rather than enzymatic alteration of lipid molecules. Similar observations have been reported in studies evaluating physicochemical properties of rendered animal fats, where tissue origin was identified as one of the major determinants of saponification value [Shahidi 2005].

The iodine value, which reflects the degree of unsaturation of fatty acids, was significantly lower in bromelain-treated samples and in pork adipose tissue treated with the higher papain dose. Since the iodine value depends primarily on fatty acid composition, these differences probably reflect selective recovery of lipid fractions differing in fatty acid profile rather than oxidative degradation during rendering. Previous studies have demonstrated that different adipose depots and animal species differ substantially in fatty acid composition, particularly in the relative proportions of saturated and monounsaturated fatty acids, which directly influence iodine value [Wood *et al.* 2008, Daley *et al.* 2010].

The peroxide value is considered one of the most important indicators of primary lipid oxidation. Despite significant differences among treatments, peroxide values remained relatively low in all rendered fats. Only bromelain-treated pork adipose tissue exhibited a significantly higher peroxide value than the remaining groups. Nevertheless, even this value was substantially lower than levels generally associated with advanced oxidative deterioration of edible fats [Shahidi and Zhong 2005]. The overall low peroxide values obtained in the present study are most likely attributable to the mild rendering conditions. Vacuum rendering at reduced pressure and relatively low temperature limits oxygen availability and decreases thermal oxidation compared with conventional atmospheric rendering. Numerous studies have demonstrated that reducing oxygen exposure during thermal processing effectively suppresses the formation of lipid hydroperoxides and secondary oxidation products [Frankel 2005, Choe and Min 2006].

Collectively, the present results demonstrate that enzymatic pre-treatment substantially improves rendering efficiency without adversely affecting the basic physicochemical quality of the rendered fat. Although some differences in acid, saponification and iodine values were observed, these changes appear to be associated primarily with differences in raw material characteristics and selective lipid recovery rather than deterioration of fat quality. Furthermore, the low peroxide values confirm that low-temperature vacuum rendering effectively limits oxidative changes, supporting the suitability of this processing technology for the production of high-quality rendered animal fats.

Color parameters

The present study demonstrated that both adipose tissue origin and enzymatic pre-treatment significantly affected the instrumental colour characteristics of rendered fats. The most pronounced changes were observed for the b^* coordinate, chroma (C) and hue angle (H), whereas lightness (L^*) remained relatively stable in beef adipose tissue following papain treatment but changed markedly in pork adipose tissue (Tab. 4).

Lightness (L^*) is considered one of the most important quality attributes of edible animal fats because it reflects the optical properties of the lipid matrix and the presence of suspended non-lipid components. Beef adipose tissue exhibited significantly higher L^* values than pork adipose tissue, irrespective of enzymatic treatment, indicating a brighter appearance of rendered beef fat. Similar differences between animal species have previously been reported and are primarily attributed to differences in adipocyte structure, connective tissue organisation and natural pigment composition rather than to processing conditions alone [Wood *et al.* 2008, Hocquette *et al.* 2010]. Interestingly, papain treatment did not significantly alter the lightness of beef fat, whereas pork adipose tissue treated with 30 mg kg⁻¹ papain exhibited a marked decrease in L^* . This observation suggests that the response of adipose tissue to proteolytic modification depends on tissue structure and species-specific characteristics.

The most pronounced effect of enzymatic treatment was observed for the b^* coordinate and chroma. Bromelain-treated samples exhibited the highest positive b^* values together with the greatest chroma, indicating a more intense yellow colour. In rendered animal fats, yellowness is influenced mainly by naturally occurring carotenoid pigments, particularly β -carotene, as well as by the optical properties of the lipid phase and the degree of light scattering [Wood *et al.* 2008]. Because neither papain nor bromelain directly modifies lipid pigments, the observed increase in b^* and chroma most likely reflects improved release of lipid fractions containing fat-soluble pigments following enzymatic disruption of connective tissue surrounding adipocytes. Enhanced liberation

Table 4. Instrumental color characteristics of various adipose tissues using proteolytic enzymes

Group	L^*	a^*	b^*	C	H
K1	80.432±1.016 ^b	-2.572±0.050 ^d	-0.730±0.235 ^f	2.680±0.108 ^f	-1.845±0.081 ^e
K2	85.948±0.031 ^a	-2.025±0.012 ^b	-0.393±0.021 ^e	2.063±0.014 ^{gh}	-1.763±0.009 ^d
P1	73.285±1.965 ^d	-2.815±0.058 ^e	-2.287±0.149 ^b	3.629±0.067 ^d	-2.253±0.040 ^g
P2	81.558±0.138 ^b	-4.955±0.055 ^f	6.633±0.082 ^c	8.280±0.097 ^c	-0.642±0.002 ^b
P3	85.600±0.009 ^a	-2.017±0.016 ^{ab}	-0.508±0.023 ^e	2.080±0.020 ^{gh}	-1.818±0.010 ^{de}
B1	81.407±0.087 ^b	-5.333±0.020 ^g	9.248±0.032 ^a	10.676±0.024 ^a	-0.523±0.003 ^a
B2	80.782±0.046 ^b	-5.315±0.005 ^g	8.755±0.029 ^b	10.242±0.026 ^b	-0.546±0.001 ^a

K1 – pork subcutaneous adipose tissue; K2 – beef subcutaneous adipose tissue; P1 – pork subcutaneous adipose tissue with 30mg/kg papain; P2 – pork subcutaneous adipose tissue with 50mg/kg papain; P3 – beef subcutaneous adipose tissue with 30mg/kg papain; B1 – pork subcutaneous adipose tissue with 30mg/kg bromelain; B2 – beef subcutaneous adipose tissue with 30mg/kg bromelain.

^{ab...} Values are reported as mean±SD; different lowercase superscript letters in the same column indicate statistically significant differences between experimental groups ($p < 0.05$).

of intracellular lipid droplets may increase pigment concentration in the rendered fat fraction without altering pigment chemistry. Colour saturation (chroma) followed a pattern similar to that observed for b^* , confirming that bromelain treatment produced the most intense colour among all experimental groups. Chroma is directly related to colour vividness and increases with greater colour purity (CIE, 2004). The substantially higher chroma values observed after bromelain treatment indicate that enzymatic pre-treatment influenced the optical characteristics of the rendered fat rather than simply changing its lightness. Similar relationships between yellowness and chroma have been reported for rendered fats and edible oils, where increased pigment concentration resulted in higher colour saturation [Frankel 2005]. Significant differences were also observed for the hue angle. Bromelain-treated samples exhibited the highest hue angle values, whereas pork adipose tissue treated with papain at 30 mg kg⁻¹ showed the lowest value. Because hue angle is derived from both a^* and b^* coordinates, these changes reflect the combined effect of reduced redness and increased yellowness observed after enzymatic treatment. The close association between b^* , chroma and hue angle suggests that enzymatic modification affected the spectral characteristics of the rendered fat primarily through changes in pigment distribution within the lipid phase rather than through oxidative discoloration. However, a potential contribution of the commercial bromelain preparation itself cannot be excluded. Although bromelain is purified from pineapple tissues, commercial enzyme preparations may differ in their degree of purification and may contain minor non-protein components originating from the plant material. An important observation is that the substantial changes in colour parameters were not accompanied by a corresponding increase in peroxide values reported in the present study. Lipid oxidation is one of the principal causes of colour deterioration in edible fats due to degradation of carotenoids and formation of secondary oxidation products [Frankel 2005, Choe and Min 2006]. However, peroxide values remained relatively low in all experimental groups, indicating that oxidative degradation was limited under the applied rendering conditions. Therefore, the observed differences in colour are unlikely to result from oxidation-induced pigment degradation but are more probably associated with differences in fat composition, tissue structure and efficiency of lipid release after enzymatic pre-treatment.

Overall, the results demonstrate that proteolytic pre-treatment modifies the visual characteristics of rendered fats while preserving low oxidative deterioration. Bromelain produced the greatest increase in yellowness and colour saturation, whereas papain treatment of beef adipose tissue maintained colour characteristics comparable to those of untreated beef fat. These findings indicate that the choice of proteolytic enzyme may influence not only rendering efficiency but also the commercial appearance of rendered animal fats, which is an important quality attribute affecting consumer perception and industrial utilisation. Since the colour and composition of the bromelain preparation used in the present study were not independently analysed, further studies are required to determine whether these components contributed to the increased b^* values observed after bromelain treatment.

Fatty acid profile in the second series of rendered animal fats

The present study demonstrated that the fatty acid composition of rendered animal fats was influenced predominantly by the origin of adipose tissue, whereas enzymatic pre-treatment resulted in comparatively smaller but statistically significant changes in selected fatty acids (Tab. 5). This observation is consistent with previous reports

Table 5. Fatty acid composition of animal rendered fat samples (% total fatty acids)

%	K1	K2	P1	P2	P3	B1	B2
C14:0	1.28±0.12 ^b	3.18±0.12 ^a	1.27±0.05 ^b	1.32±0.08 ^b	2.67±0.10 ^{ab}	1.27±0.05 ^b	2.72±0.15 ^{ab}
C14:1	0.15±0.05 ^{bc}	0.63±0.05 ^{ab}	0.15±0.05 ^{bc}	0.13±0.05 ^c	0.65±0.10 ^a	0.15±0.05 ^{bc}	0.68±0.10 ^a
C15:0	1.13±0.05 ^a	1.13±0.10 ^a	1.22±0.08 ^a	1.17±0.12 ^a	1.17±0.05 ^a	1.10±0.06 ^a	1.15±0.10 ^a
C16:0	27.08±0.80 ^a	28.17±1.17 ^a	23.50±0.84 ^b	22.83±0.75 ^b	25.17±0.41 ^{ab}	23.00±0.89 ^b	24.3±0.52 ^{ab}
C16:1 cis-9	2.62±0.23 ^a	2.40±0.24 ^a	2.48±0.37 ^a	2.65±0.24 ^a	2.45±0.35 ^a	2.72±0.19 ^a	2.58±0.27 ^a
C17:0	0.17±0.08 ^b	1.05±0.05 ^{ab}	0.25±0.05 ^{ab}	0.17±0.08 ^b	1.10±0.09 ^a	0.17±0.08 ^b	1.13±0.08 ^a
C17:1	0.17±0.08 ^c	0.55±0.05 ^{ab}	0.27±0.05 ^{bc}	0.25±0.05 ^{bc}	0.55±0.08 ^{ab}	0.25±0.05 ^{bc}	0.62±0.04 ^a
C18:0	12.25±1.08 ^c	21.53±0.77 ^a	12.00±0.89 ^c	11.83±0.75 ^c	19.25±0.76 ^b	11.83±0.52 ^c	19.3±0.82 ^b
C18:1 cis-9	43.08±1.11 ^{abc}	36.00±0.89 ^c	44.17±1.17 ^{ab}	45.58±1.02 ^a	39.33±0.61 ^{bc}	44.58±0.66 ^{ab}	39.83±0.75 ^{bc}
C18:1 trans-11	<0.10	1.63±0.15 ^b	<0.10	<0.10	2.22±0.08 ^a	<0.10	2.20±0.09 ^a
C18:2 n-6	10.50±1.05 ^b	1.72±0.08 ^d	13.40±0.80 ^a	12.67±0.82 ^a	2.68±0.12 ^c	13.33±0.82 ^a	2.67±0.14 ^c
C18:2 trans	<0.10	0.33±0.05 ^a	<0.10	<0.10	0.32±0.04 ^a	<0.10	0.32±0.04 ^a
C18:2 cis-9, trans-11	<0.10	0.63±0.15 ^{ab}	<0.10	<0.10	1.17±0.05 ^a	<0.10	1.07±0.08 ^a
C18:3 n-6	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
C18:3 n-3	0.35±0.08 ^b	0.43±0.05 ^{ab}	0.40±0.06 ^{ab}	0.35±0.05 ^b	0.53±0.05 ^a	0.43±0.08 ^{ab}	0.52±0.08 ^a
C20:0	0.28±0.04 ^a	0.22±0.04 ^a	0.27±0.05 ^a	0.22±0.04 ^a	0.25±0.05 ^a	0.2±0.05 ^a	0.25±0.05 ^a
C20:1 n-9	0.57±0.05 ^a	0.25±0.05 ^c	0.57±0.05 ^a	0.55±0.05 ^{ab}	0.33±0.05 ^{bc}	0.55±0.05 ^{ab}	0.35±0.05 ^{abc}
C20:2	0.25±0.05 ^a	0.23±0.05 ^a	0.27±0.05 ^a	0.27±0.05 ^a	0.25±0.05 ^a	0.23±0.05 ^a	0.27±0.05 ^a
SFA	42.20±0.39 ^{abc}	55.28±0.96 ^a	38.50±1.29 ^{bc}	37.53±0.96 ^c	49.60±0.46 ^{ab}	37.63±1.14 ^c	48.92±0.90 ^{abc}
MUFA	46.63±1.04 ^{abc}	41.47±0.91 ^c	47.68±1.06 ^{ab}	49.2±0.93 ^a	45.53±0.55 ^{bc}	48.30±0.66 ^{ab}	46.27±0.82 ^{abc}
PUFA	11.25±1.03 ^b	3.40±0.16 ^d	14.22±0.80 ^a	13.43±0.83 ^a	5.00±0.12 ^c	14.15±0.79 ^a	4.88±0.16 ^c

K1- Pork subcutaneous adipose tissue, K2- Beef subcutaneous adipose tissue, P1- Pork subcutaneous adipose tissue with 30mg/kg papain, P2- Pork subcutaneous adipose tissue with 50mg/kg papain, P3- Beef subcutaneous adipose tissue with 30mg/kg papain, B1- Pork subcutaneous adipose tissue with 30mg/kg bromelain, B2- Beef subcutaneous adipose tissue with 30mg/kg bromelain, a-c- values are reported as mean±SD different letters in the same line indicate statistically significant differences ($p < 0.05$).

indicating that species, adipose tissue depot and feeding system are the principal determinants of fatty acid composition in animal fats, whereas processing generally exerts a limited effect on the overall lipid profile [Wood *et al.* 2008, Daley *et al.* 2010, Stelmasiak *et al.* 2019].

Beef-derived rendered fats contained significantly higher proportions of saturated fatty acids (SFA) than pork-derived fats. The greatest differences were observed for palmitic (C16:0) and stearic (C18:0) acids, which together accounted for the majority of SFA in all beef samples. This finding agrees with previous studies demonstrating that ruminant adipose tissue is characterised by higher concentrations of saturated fatty acids owing to extensive ruminal biohydrogenation of dietary unsaturated fatty acids before intestinal absorption [Wood *et al.* 2008, Scollan *et al.* 2006]. In contrast, pork adipose tissue contained substantially lower proportions of SFA and correspondingly higher concentrations of unsaturated fatty acids, reflecting the absence of ruminal biohydrogenation in monogastric animals [Wood *et al.* 2008]. Oleic acid (C18:1 *cis*-9) was the predominant fatty acid in all rendered fats, accounting for approximately 36-46% of total fatty acids. Its high proportion is consistent with numerous reports describing oleic acid as the principal monounsaturated fatty acid in both pork and beef adipose tissue [Wood *et al.* 2008, Daley *et al.* 2010]. The significantly higher oleic acid concentration observed in pork fat compared with beef fat contributed directly to the higher total MUFA content in pork-derived rendered fats.

One of the most characteristic findings of the present study was the exclusive presence of *trans*-vaccenic acid (C18:1 *trans*-11) and conjugated linoleic acid (CLA; C18:2 *cis*-9, *trans*-11) in beef-derived rendered fats. These results are fully consistent with the well-established metabolism of ruminants, in which ruminal microorganisms convert dietary linoleic and α -linolenic acids into vaccenic acid through biohydrogenation. Subsequently, vaccenic acid is converted to rumenic acid (*cis*-9, *trans*-11 CLA) by Δ^9 -desaturase in animal tissues [Scollan *et al.* 2006]. Consequently, ruminant fats naturally contain appreciable concentrations of vaccenic acid and CLA, whereas these fatty acids are virtually absent from pork fat.

The significantly higher concentrations of linoleic acid (C18:2 *n*-6) and the higher total PUFA content observed in pork-derived rendered fats also agree with previous investigations comparing ruminant and monogastric species. Because pigs incorporate dietary fatty acids into adipose tissue with relatively limited modification, pork fat generally contains considerably greater proportions of linoleic acid and other polyunsaturated fatty acids than beef fat [Wood *et al.* 2008, Dugan *et al.* 2011]. Conversely, the low PUFA content observed in beef fat results from microbial hydrogenation of unsaturated fatty acids in the rumen before absorption [Scollan *et al.* 2006]. Although enzymatic pre-treatment produced statistically significant differences in several individual fatty acids, its influence on the overall fatty acid profile was relatively limited compared with the effect of animal species. Neither papain nor bromelain possesses lipolytic activity capable of directly modifying fatty acid composition. Instead, both enzymes hydrolyse structural proteins within

the extracellular matrix of adipose tissue, facilitating lipid release during rendering [Arshad *et al.* 2016]. Therefore, the observed differences are more likely attributable to selective recovery of lipid fractions associated with different adipocyte populations or connective tissue structures rather than enzymatic modification of lipid molecules themselves. Interestingly, enzymatic treatment of beef adipose tissue increased the proportions of vaccenic acid and CLA compared with untreated beef fat. Since both compounds are naturally synthesised in ruminant tissues before slaughter, the increase observed after rendering most likely reflects more efficient recovery of lipid fractions enriched in these fatty acids rather than *de novo* formation during processing. Similar conclusions have been drawn in studies evaluating technological processing of ruminant fats, where differences in lipid fraction recovery influenced the relative abundance of individual fatty acids without altering their biochemical origin [Wood *et al.*, 2008]. Also preservation of the characteristic fatty acid profile may be related to the relatively mild conditions applied during vacuum rendering. The process was conducted at $95\pm 2^\circ\text{C}$ under reduced pressure (200 mbar), thereby limiting exposure of the lipid fraction to atmospheric oxygen. From a nutritional perspective, the higher concentrations of vaccenic acid and CLA in beef-derived rendered fats may represent an additional quality advantage. Vaccenic acid serves as the primary endogenous precursor of rumenic acid in humans through $\Delta 9$ -desaturase activity, while CLA has been associated with several potential biological effects, including modulation of lipid metabolism and antioxidant and anti-inflammatory activity, although the magnitude of these effects depends on dietary intake and remains under investigation [Dilzer and Park 2012]. In contrast, the higher PUFA content observed in pork fat may contribute positively to nutritional quality but may also increase susceptibility to lipid oxidation because polyunsaturated fatty acids are more readily oxidized than saturated or monounsaturated fatty acids [Frankel 2005].

Overall, the present findings demonstrate that the fatty acid composition of rendered fats is determined primarily by animal species rather than by enzymatic pre-treatment. Nevertheless, proteolytic treatment improved rendering efficiency while maintaining the characteristic fatty acid profile of the respective raw materials, indicating that papain and bromelain can be applied to increase fat recovery without substantially altering the nutritional composition of the rendered fat.

Conclusions

Enzymatic pre-treatment of adipose tissue with papain and bromelain significantly improved the efficiency of low-temperature vacuum rendering. Both enzymes increased rendering yield and the fat-to-crackling ratio compared with untreated controls, demonstrating that proteolytic modification of adipose tissue before rendering enhances lipid recovery. Papain proved to be the most effective proteolytic enzyme under the applied processing conditions. Beef subcutaneous adipose tissue treated with 30 mg kg^{-1} papain achieved the highest rendering yield (66.85%) and

the highest fat-to-crackling ratio (2.91), indicating the greatest process efficiency among all experimental groups. Enzymatic pre-treatment did not adversely affect the physicochemical quality of the rendered fats. Despite significant differences in acid, saponification, iodine and peroxide values, all analysed fats exhibited low acid and peroxide values, indicating limited hydrolytic and oxidative deterioration during low-temperature vacuum rendering. The origin of adipose tissue had a greater influence on the physicochemical properties and fatty acid composition of rendered fats than enzymatic treatment. Beef-derived fats contained higher proportions of saturated fatty acids, stearic acid, vaccenic acid and conjugated linoleic acid (CLA), whereas pork-derived fats were characterised by higher contents of oleic acid and polyunsaturated fatty acids. Instrumental colour parameters were significantly influenced by enzymatic pre-treatment. Bromelain produced the greatest increase in yellowness (b^*), chroma and hue angle, whereas papain treatment preserved the colour characteristics of beef fat close to those of untreated samples. These changes occurred without evidence of excessive lipid oxidation. The fatty acid profile remained characteristic of the respective animal species following enzymatic pre-treatment, indicating that papain and bromelain improved fat recovery without fundamentally altering the nutritional composition of the rendered fats. The combined application of enzymatic pre-treatment and low-temperature vacuum rendering represents a promising technological approach for improving the efficiency of animal fat recovery while maintaining desirable physicochemical quality, colour stability and nutritional value. The results indicate that papain, in particular, has considerable potential for industrial application in the production of high-quality rendered animal fats.

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