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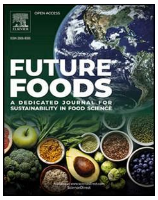

Upcycling supercritical-CO_2-defatted tiger nut milk Co-products into pork burgers: A sustainable fat replacer with enhanced quality properties

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ABSTRACT

Horchata is a traditional beverage from Valencia, Spain, made from tiger nuts (a tuber), which industrial production generates large amounts of co-products. These co-products are rich in several compounds (i.e. oil), which can be extracted by applying green technologies such as supercritical-CO_2. However, even after oil extraction, some co-products remain. Following the principles of a circular economy, these remaining co-products can be reintroduced into the food chain as fat replacers in meat products. The study aimed to develop pork burgers with different concentrations of supercritical-CO_2 (29.5 MPa, 45.8 °C) defatted tiger nut milk co-products (DTNC) and evaluate their stability, chemical composition, and physical properties. DTNC are rich in fiber and minerals, and their addition to pork burgers resulted in reduced fat content while increasing fiber, magnesium, and potassium in a concentration-dependent manner. The DTNC improved the cooking properties of the burgers and significantly affected their color and texture. Although the presence of the defatted tiger nut co-products reduced the scores for most of the sensory attributes, when added up to 3 %, all of them were rated higher than 5. At higher concentrations, the lowest values were obtained for granularity, juiciness and crumbliness. In conclusion, replacing backfat with up to 3 % DTNC in pork burgers provides a method to reduce fat while increasing fiber and mineral content, all while maintaining the expected qualities of the burger.

1. Introduction

Tiger nut (*Cyperus esculentus* L.) is a tuber whose cultivation dates back to the time of the ancient Egyptians. From Egypt, the cultivation of the tiger nut spread to North Africa (where countries such as Niger, Burkina Faso and Mali are the main producers, reaching 10,000 Tn yearly), towards the Iberian Peninsula and Sicily with the Islamic waves of the Middle Ages. The Islamic culture expanded the cultivation of tiger nuts in the Mediterranean areas of Spain, mainly in the Valencian Community (RCPDO tiger nut of Valencia, 2024). In recent years, global interest in tiger nuts has increased due to their status as a 'superfood', comparable to others like quinoa and chia, because of their exceptional bioactive compounds, biological activities, and nutritional and health benefits (Bezerra et al., 2023). They are rich in dietary fiber and fat, mainly monounsaturated fatty acids, especially oleic acid, minerals such as potassium and phosphorus, vitamins E and C, and polyphenolic compounds (flavonoids, tannins, etc.) with interesting antioxidant properties (Bezerra et al., 2023; Zhang and Sun, 2023). Tiger nuts have been found beneficial in reducing risk of cardiovascular disease, obesity, constipation and colon cancer (Samuel et al., 2024; Yu et al., 2022). The most widely understood uses of the tiger nut worldwide are to obtain flour for biscuits, pasta or chocolate base, as well as oil extraction, and other pharmaceutical and cosmetic uses. However, in the Valencian Community of Spain, nearly the entire production of tiger nuts (8,000 Tn) is covered by the European Union's protected designation of origin (PDO) known as 'Chufa de Valencia' (DOGV- Consejo Regulador PDO chufa de Valencia, 2010). These tiger nuts are used to produce a traditional beverage called 'horchata de chufa' (tiger nut milk). The

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manufacturing process of "horchata de chufa" involves rehydrating the tubers by immersing them in water. The tubers are then crushed, and more water is added (approximately 3 L of water per kg of dried tiger nut) to facilitate the process. It is left to macerate for a while and then sieved to separate the liquid from the solid residue. Subsequently, sugar is added, and it is refrigerated close to 0°C to obtain the "horchata" (tiger nut milk). During this process, the co-products generated (solid residue) can represent up to 60 % of the original plant material (tiger nuts) and their management is both an economic and environmental problem for the tiger nut industry. These co-products are rich in oil, dietary fiber (mainly insoluble dietary fiber), minerals and polyphenolic compounds (Pelegrín et al., 2022; Razola-Díaz et al., 2022; Roselló-Soto et al., 2018; Sánchez-Zapata et al., 2009). Due to their composition and techno-functional properties (Sánchez-Zapata et al., 2009; Sánchez-Zapata, Fernández-López, et al., 2013) they have been successfully used as ingredients for the food industry (Bezerra et al., 2023; Oladunjoye and Alade, 2024), specially for the meat industry either as source of dietary fiber, as functional ingredient or even as freezing protectant (Sánchez-Zapata et al., 2010, 2013). In addition to the direct use of these co-products as a food ingredient, they have also been subjected to different extraction processes to obtain extracts rich in specific components with food, cosmetic or pharmaceutical interest (Roselló-Soto et al., 2019a, 2019b, 2019c).

Tiger nut oil is one of the components of co-products that has aroused the most interest due to its multiple applications in food and cosmetics as well as its use as biodiesel. In this regard, innovative and green approaches have emerged in view of the sustainable conversion of discarded co-products to oil. More specifically, supercritical-CO_2 extraction seems to be a practicable solution considering the environmental, economic, and social benefits that this technology entails, for instance, the low price, low toxicity and high availability of CO_2. In addition, supercritical fluids at high pressures exhibit intermediate properties between liquids and gases. This unique state enhances mass transfer and solvent penetration, allowing efficient recovery of target molecules. As a result, the extraction process is rapid and environmentally friendly compared to traditional methods, which are often time-consuming and harmful due to the use of organic solvents like hexane or chloroform (Salgado-Ramos et al., 2023a, 2023b). After the oil extraction from the tiger nut co-products, a solid residue would remain which could also be useful as a food ingredient, in line with the principles of the 2030 Agenda for Sustainable Development and the objective to promote food circular systems. This involves designing processes that completely minimize waste and maximize resource utilization across the entire food value chain. In this case, the "waste" is minimized by its valorization, upcycling these co-products into new meat products. Burgers are a widely consumed product and therefore their reformulation to make them healthier is of great interest. Lately, most efforts are being directed towards reducing their fat content (mainly saturated fats) or modifying their lipid profile (increasing unsaturated fats), due to the link between high dietary saturated fat intake and health problems such as diabetes, obesity, and cardiovascular diseases. Different strategies can be used to achieve this purpose, such as replacing them with ingredients rich in dietary fiber, with the advantage to meet two dietary recommendations for today's population: reducing saturated fat intake and increasing dietary fiber intake.

This work aimed to evaluate the feasibility of using this new food ingredient (supercritical-CO_2-defatted tiger nut milk co-product) in pork burgers as a partial animal fat replacer, and to study its effect on their technological, nutritional, and sensory properties.

2. Materials and methods

2.1. Raw materials

Fresh tiger nut co-products were supplied by an "horchata" manufacturing industry FARTONS POLO (Alboraya, Valencia, Spain). Meat ingredients (pork lean meat and backfat were purchased from a local butchery (Murcia, Spain) and the additives and spices were provided by an authorized supplier of food ingredients (Suministros River S.L.U., Alicante, Spain)

2.2. Processing of fresh tiger nut co-products

The discarded solid co-products from the horchata industry, specifically from the initial pressing of tiger nuts, were dried at 40-45°C. The dried residue, with a moisture of around 6-7 % and particle size distribution ø>1.6 mm, was subsequently processed through supercritical fluid extraction (SFE) at 29.5 MPa pressure and 45.8°C temperature. No doping organic cosolvents were utilized, being the process conducted with pure CO_2. CO_2 was recirculated after processing to being used in successive extractions. An orange, oily crude was directly recovered along with a remaining solid. This Remaining solid was called supercritical-CO_2-defatted tiger nut milk co-product (DTNC) and was used in the current work as an ingredient in pork burgers.

2.3. Characterization of the supercritical-CO_2-defatted tiger nut milk co-products (DTNC)

2.3.1. Chemical composition

Chemical composition of both raw materials, the fresh tiger nut milk co-product and the DTNC, was determined. The analysis of fibers (cellulose, hemicellulose and lignin), hexane extractable fractions (non-polar accessible fractions) and water extractable fractions (polar accessible fractions) was carried out followed the methodology based on the standard procedures described by Sluiter et al. (2008), with the modifications described by Salgado-Ramos et al. (2022). Proteins were determined by the Kjeldahl method. Mineral profile was determined on DTNC using inductively coupled plasma–mass spectrometry (ICP-MS) Shimadzu MS-2030 (Shimadzu, Kioto, Japan) with the following operation conditions: nebulizer gas flow, 0.91 L/min; radio frequency 1200 W lens voltage 1.6 V; cool gas 12.0 L/min; auxiliary gas 0.70 L/min, previously described by Muñoz-Bas et al. (2024). Before mineral determination, samples were digested with nitric acid (67 %) and peroxide (33 %) in a microwave. The results were expressed as mg or µg per 100 g of sample.

2.3.2. Particle size determination

DTNC (100 g) were passed through different sieves with the following mesh sizes of 1651, 1168, 701, 417 and 210 µm. After five minutes of agitation, the weight of the DTNC in each sieve was recorded. The results were expressed as the percentage of different particle sizes.

2.3.3. Physicochemical properties

The water activity (aw) was determined at 25°C using an electric hygrometer NOVASINA TH200 (Novasina; Axair Ltd., Pfaeffikon, Switzerland). The pH was measured in a water solution (1:10 solid: liquid ratio g/mL) after 10 minutes of magnetic agitation with a pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain). The color was evaluated with a spectrophotocolorimeter Minolta CM-700 (Minolta Camera Co., Osaka, Japan) using Observator 10^o, Illuminant D65, 11 mm aperture of the instrument for illumination, and 8 mm for measurement. CIELab color space was selected to obtain the color coordinates: lightness (L*), redness (a*) and yellowness (b*), from which croma (C*), hue (h*) and color differences (compared to control sample) were calculated.

2.3.4. Techno-functional properties

The following techno-functional properties were assessed: Water holding capacity (WHC), Swelling capacity (SW), Oil holding capacity (OHC), emulsion ability (EA) and emulsion stability (ES) following the methodology described by Lucas-González et al. (2017).

2.4. Pork burger manufacture

The burgers were prepared in the food pilot plant of the Miguel Hernández University (Orihuela, Spain) following normal industrial processing methods. Four batches (2 Kg each one) were carried out: control batch (CB), without the addition of defatted tiger nut co-products, burgers with 3 % of defatted tiger nut co-products (BTN-3), burgers with 6 % of defatted tiger nut co-products (BTN-6), and burgers with 9 % of defatted tiger nut co-products (BTN-9). The control burger ingredients were pork lean (70 %), backfat (30 %), water (7 %), salt (1.5 %), pepper (0.05 %) and garlic (0.05 %). For the other formulations, part of the backfat was replaced by the corresponding percentage of tiger nut added in each formulation (Supplemented Table 1). Furthermore, the BTN-9 needed more water (9 %) to tolerate the 9 % of DTNC. Fresh lean pork meat and backfat were obtained from a local market (Orihuela, Spain). Initially, pork, salt, pepper, garlic and cool water were mixed to obtain a homogenized meat dough. Then, the meat dough was divided into four batches, and the corresponding amount of backfat or tiger nut co-products was added. The burger dough was mixed. Burgers of 25 g were formed with a manual burger-forming machine. The burgers were packed in plastic bags with zip closures and refrigerated (4 °C). The process was repeated twice on different days.

2.5. Pork burgers characterization

2.5.1. Chemical composition

The proximate composition of burgers (moisture, fat, ash and protein) was determined following the corresponding AOAC methods (AOAC, 2007). Total dietary fiber (TDF) and insoluble dietary fiber (IDF) were measured following AOAC methods using a Total Dietary Fiber Assay Kit (Sigma- TDF100A). Mineral profile was determined in the same way as described in section 2.3.1.

2.5.2. Physicochemical properties

The pH and color were determined in a raw and cooked burger, while water activity was measured in raw burgers. The pH was analyzed with a Hach puncture electrode probe (5233) connected to a pH meter (model SensIONTM + pH3, Hach-Lange S.L.U., Vésenaz, Switzerland). At the same time, color and water activity were determined in the same way as described in section 2.3.3.

2.5.3. Texture

Texture profile analysis (TPA) was performed on raw and cooked pork burgers with a Texture Analyser TA-XT2i (Stable Micro Systems, Surrey, UK). The samples were cut in a cube (1 cm3) and subjected to a 2-cycle compression to 75 % original height with a cylindrical probe of 10 cm diameter using a compression load of 25 kg, with a speed of 5 mm/s and at 20–25°C. Cohesiveness, hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience were calculated as described by Bourne (1978).

Table 1
Chemical composition (g / 100g dry matter) of tiger nut milk co-products before and after supercritical-CO_2 fluid extraction.

	TNC	DTNC
Extractives		
Non-polar accessible fraction	11.46 ± 0.69	3.23 ± 1.00
Polar accessible fraction	3.24 ± 0.99	4.04 ± 0.69
Fibres		
Holocellulose	24.38 ± 2.00	50.37 ± 3.57
Cellulose	-	21.06 ± 2.25
Hemicellulose	-	29.34 ± 1.35
Lignin	28.96 ± 3.83	34.55 ± 5.38
Proteins	3.34 ± 0.49	4.44 ± 0.35
Others	28.62 ± 7.01	5.40 ± 0.71

TNC: Tiger nut milk coproducts; DTNC: Supercritical-CO_2-defatted tiger nuts milk coproducts

2.5.4. Microbiological analysis

The microbiological quality of burgers was studied following the techniques described by the American Public Health Association (APHA, 2015). Microbial contents of mesophilic aerobic bacteria, *Enterobacteriaceae*, yeast, molds and lactic acid bacteria. were evaluated. Except for lactic acid bacteria, which were grown using a traditional plate count agar method (MRS-agar medium), the other microorganisms were grown using their corresponding petrifilms® media and the results were expressed as log colony forming units (CFU)/g of pork burger.

2.5.5. Cooking properties

The cooking quality of the burgers was studied through three determinations: cooking loss, which measures the fluids loss during the cooking process; shrinkage, which evaluates the reduction in diameter of burgers after cooking; and thickness increase, which refers to the increased density of the burger after cooking. The formula of those properties can be seen in equations 1, 2 and 3, respectively (Lucas-González et al., 2020). The values were expressed as percentages of three independent burgers. The cooking process was standardized for all burger formulations. The raw burgers were cooked for three minutes on each side on a hot griddle. The core of burgers reaches 72 °C. The burgers were left for 15 minutes to cool at room temperature before analyses. Fig. 1 shows raw and cooked burgers (control and DTNC-added).

$$Cooking\ loss\ (\%) = \left(\frac{Weight\ raw\ burger - Weight\ cooked\ burger}{Weight\ raw\ burger}\right) \times 100 \tag{1}$$

$$Shrinkage\ (\%) = \left(\frac{Diameter\ raw\ burger - Diameter\ cooked\ burger}{Diameter\ raw\ burger}\right) \times 100 \tag{2}$$

$$Thickness\ increase\ (\%) = \left(\frac{Thickness\ raw\ burger - Thickness\ cooked\ burger}{Thickness\ cooked\ burger}\right) \times 100 \tag{3}$$

2.5.6. Lipid oxidation

Secondary products of lipid oxidation were measured in the raw and cooked pork burgers using the Thiobarbituric Acid Reactive Substances Assay (TBARS), following the methodology of Rosmini et al. (1996) with slight modifications applied by Lucas-González et al. (2019). In brief, 2 or 5 g of cooked and raw burgers were mixed with 10 mL of Trichloroacetic acid (10 %). After 15 minutes of agitation bath (100 rpm), the samples were left in a freezer (15 minutes). The samples were vacuum filtered and 2 mL of filtered sample was mixed with 2 mL of thiobarbituric acid (0.5 %). After 35 minutes in a boiling bath (95 °C) the samples were cooled in an ice bath and the absorbance at 535 nm was measured. A blank with trichloroacetic and thiobarbituric acids was used as a spectrophotometric blank. The results were expressed as mg malonaldehyde (MDA)/Kg sample.

2.5.7. Sensory evaluation

A hedonic analysis (nine-point hedonic scale) was carried out. Pork burgers were cooked at the same conditions described in section 2.5.5 The taste test took place in the tasting room of Miguel Hernández University (Orihuela, Alicante, Spain). Fifty non-training habitual consumers of burgers participated in the sensory analysis. The sex of participants was 54 % feminine, 44 % masculine and 2 % non-binary. The age range of participants was 18-25 years 56 %; 26-30 years 14 %; 31-35 years 8 %; 36-40 years 4 %; 41-50 years 4 %; 51-60 years 14 %. The participants evaluated 7 sensorial attributes: general aspect, color,

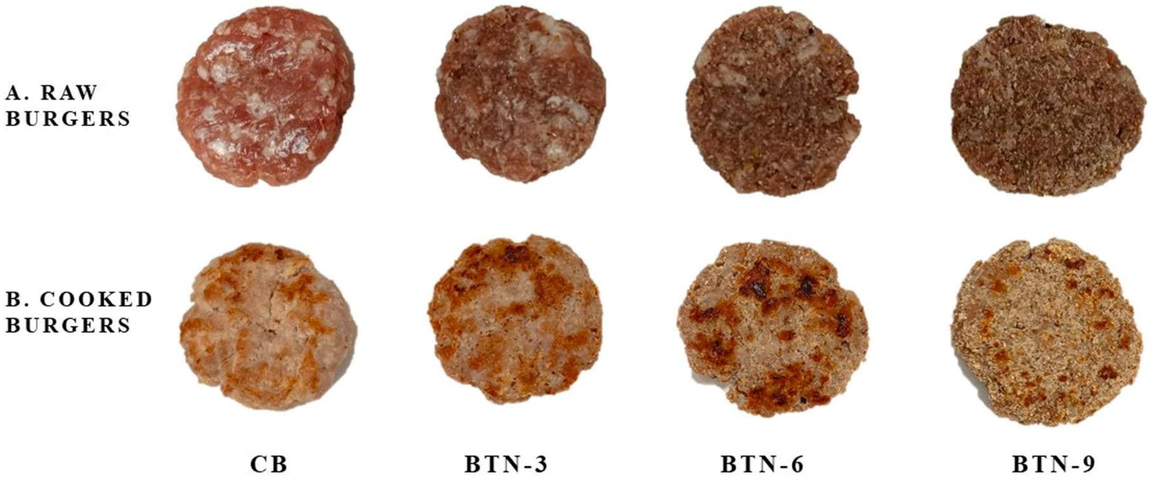

Fig. 1. A Raw pork burgers and B. Cooked pork burgers without DTNC (CB) and with the addition of DTNC.
DTNC: supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9% of DTNC.

hardness, juiciness, crumbliness, flavor and granularity. The attribute scale was from 1 (dislike extremely) to 9 (like extremely). At the end of the test, panelists were asked to give a score for general acceptability of the product from 0 to 9. For the sensory analysis, all the participants gave informed consent via the statement "I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for sensory evaluation.

2.6. Statistical analysis

The results were expressed as mean ± SD of three independent measures. SPSS (IBM SPSS Statistics version 26) was used to carry out an ANOVA (one-way) assay. Tukey's post host test was used to expose significant differences when the p-value was <0.05.

3. Results and discussion

3.1. Characterization of supercritical-CO_2-defatted tiger nut milk co-product (DTNC)

Chemical composition results of tiger nut milk co-products before and after supercritical-CO_2 are shown in Table 1. After supercritical CO_2 extraction, the tiger nut milk co-products increased their content of fiber, especially in holocellulose, which increased by 108 % its content. Also, their protein and lignin content increased after the extraction process but in a more discrete way 32 and 18 %, respectively. The increase of these nutrients is linked with the reduction of fat (around 72%) and other compounds (around 82%), that may include primary metabolites such as polyphenols, as well as intrinsic water. This fact is explained since both substances are highly prone to be removed by processing with supercritical-CO_2 (Pravallika et al., 2023; Roselló-Soto et al., 2019b). Nonetheless, polyphenols acting as bioactive molecules are present in the recovered oil, providing a notable antioxidant activity to this crude which can result in interest for application in alternative, healthier foods. Therefore, after supercritical CO_2 extraction of tiger nut milk co-products, an oil rich in polyphenols and DTNC rich in fiber have been obtained.

As the DTNC was the ingredient used in the formulation of pork burgers, it was completely characterized before its incorporation into the meat matter to plan the range of percentages at which they could be incorporated, in view of their feasibility both nutritionally and technologically.

Regarding mineral content (Table 2), the main minerals present in DTNC were potassium, calcium and phosphorus. Nevertheless, the most remarkable result was the contribution to daily reference intakes of minerals. Except for potassium, the contribution was higher than 15 % of the rest of the minerals, highlighting the content of copper, manganese and magnesium, which contribute to 57.3, 40.6 and 33.2 % of daily reference intakes, respectively (REGULATION (UE) No 1169/2011).

Table 2
Physicochemical and techno-functional properties and mineral profile of supercritical-CO_2-defatted tiger nut milk co-product.

Mineral profile	Ca (mg / 100 g)	211.43 ± 4.00
	Cu (mg / 100 g)	0.57 ± 0.00
	Fe (mg / 100 g)	3.26 ± 0.62
	K (mg / 100 g)	287.99 ± 7.97
	Mg (mg / 100 g)	124.63 ± 3.94
	Mn (mg / 100 g)	0.81 ± 0.01
	Na (mg / 100 g)	90.87 ± 2.59
	P (mg / 100 g)	129.51 ± 0.43
	Zn (mg / 100 g)	2.59 ± 0.07
Physicochemical properties	pH	5.37 ±0.03
	aw	0.328 ±0.005
	L*	75.52 ±2.36
	a*	2.42 ±0.64
	b*	14.42 ±0.84
	C*	14.63 ±0.86
	h*	80.51 ±2.31
Techno-functional properties	WHC (g/g)	8.90 ± 0.09
	OHC (g/g)	5.92 ± 0.31
	SWC (mL/g)	2.91 ± 0.58
	EC (mL/mL)	3.83 ±0.71
	ES (%)	81.94 ±19.49

WHC: Water holding capacity; OHC: Oil holding capacity; SWC: Swelling capacity; EC: Emulsion capacity; ES: emulsion stability.

The particle size of flour or powders used as food ingredients directly affects the final product's texture, consistency, and appearance (Dussán-Sarria et al., 2019). In the case of meat products, the particle size of the incorporated flours or co-products mainly influences their water absorption capacity, the formation of the meat batter, and their behavior during cooking. Regarding the particle size of DTNC, more than 56% showed a particle size between 417 and 1168 μm (28.5% between 701-1168 μm, and 28.3% between 417-701 μm), 28.6% showed a particle size higher (18.6% between 1168 and 1651 μm and 10 % > 1651 μm) and 14.6 % lower (12.0 % between 210 and 417 μm and 2.6 % < 210 μm). It has been reported that flours with high uniformity of granulometry promote better sensory quality of texture, taste, and visual appearance of the final product, as it absorbs water homogeneously, promoting uniform cooking (Dussán-Sarria et al., 2019). However, in this case, we decided to use the complete DTNC without separating the different size fractions or subjecting it to milling to avoid further manipulations, which can generate other co-products or processes that could alter its specific physicochemical and techno-functional characteristics (Lucas-González et al., 2017).

The physicochemical and techno-functional properties of DTNC are shown in Table 2. The pH of the DTNC is an important parameter that could determine its suitability for incorporation into specific food matrices. In this case, as the pH is near neutrality, it would seem ideal for a neutral food matrix as meat products. Additionally, the pH of these rich-fiber ingredients may affect their interaction with other nutrients during digestion (López-Marcos et al., 2015a). Water activity plays an essential role in food safety. As it was expected (as a powder or flour ingredient), the water activity of DTNC allows its classification as a low-moisture foods (aw< 0.60), which means that it can be considered microbiologically safe due to the limited availability of water (Karuppuchamy et al., 2024). Color is one of the most important parameters in food ingredients because of the potential impact on the final food color, which directly affects consumers. L* values of DTNC are into the range of lightness values reported for fiber-rich extracts from fruit co-products (López-Marcos et al., 2015b; Silva et al., 2022), and in the case of being incorporated into fresh raw materials, no significant changes in its lightness are foreseen. The values of the a* and b* coordinates of fiber-rich ingredients are highly dependent on the structural integrity of the fiber and the pigment content from the raw material. In this case, DTNC showed a* and b* values in the range of those reported for fresh tiger nut co-products ($a^* = 2.17$ and $b^* = 17.11$; Sánchez-Zapata et al. 2009), which would indicate its suitability in a large variety of food matrices, especially in meat and fish products, where no extreme color changes are expected. Unlike other rich-fiber co-products from red, orange, or blue fruits, whose intense colors limit their application to various food matrices. The hue value of DTNC is in the range of orange-yellowish.

The hydration properties, such as water-holding capacity (WHC) and swelling capacity (SWC), give information about the behavior of the fiber-rich co-products in a particular food system, which is essential for the development of foods. These properties of fiber rich co-products are affected by particle size, structure and chemical composition (Lucas-González et al., 2017), which limit the amount that can be added to the final product. The DTNC showed a WHC 8.9 times its weight. WHC values between 2.3 and 17.0 (g water/g sample) have been reported for agro-industry co-products (Sánchez-Zapata et al., 2009). The treatment applied to obtain co-products could result in the release of free hydroxyl groups, increasing its surface area and, in turn, the WHC by opening its compact structure (Silva et al., 2022). SWC refers to the change in volume of the sample as it absorbs water. In this case, DTNC exhibited a low SWC, which could be related to the low content of soluble fibers. In contrast, DTNC presented an oil-holding capacity (OHC) which was higher than other co-products (Sánchez-Zapata et al., 2009). This higher capacity could be attributed to its content of insoluble fibre. The OHC of power ingredients depends on the surface characteristics, total charge density and hydrophobicity of the particles (Meng et al., 2019). Additionally, this property is associated with flavor retention and cooking yield, both of which are considered beneficial attributes for cooked meat products. Chen et al. (2019) reported that when lignocellulosic materials are subjected to different treatments (chemical and physical), larger porosity and surface are obtained resulting in increased WHC, SWC and OHC. Emulsion capacity and emulsion stability are techno-functional properties interesting for the development of foods with two immiscible components (fat and water). DTNC showed low EC but high ES values, compared with other rich-fiber co-products (Sánchez-Zapata et al., 2009). High EC values are related to the interaction of protein and soluble fibers (Silva et al., 2022), but the content of both in DTNC is low which resulted in low EC values.

3.3. Chemical composition of pork burgers

Table 3 shows the chemical composition of pork burgers reformulated with DTNC. Replacing pork backfat with DTNC affected the fat content of both raw and cooked burgers, while the other components (protein, ash and moisture) were not modified ($p > 0.05$). The fat content decreased in the raw pork burgers in a concentration-dependent manner, as can be expected. The higher the DTNC, the less the fat content ($p < 0.05$). Instead, the fat substitution in burger formulations for DTNC promoted the increase in four of the nine minerals quantified in studied burgers (Table 3). Those were calcium, manganese, copper and magnesium ($p < 0.05$). Magnesium increased in burgers in a concentration-dependent manner ($p < 0.05$). Ca and Mg amounts were only modified in pork burgers concerning control burgers (without DTNC) on BTN-9 ($p < 0.05$). Regarding copper concentration, the three formulations with DTNC showed significant differences with CB ($p < 0.05$). Those results are in concordance with the mineral content reported in the DTNC. Additionally, it emphasizes that DTNC can serve as mineral concentrate to enhance minerals in another food matrix. Incorporating non-meat ingredients into burgers has been previously reported to contribute to modifying the amount of burger minerals, such as in burgers where salt was 75 % substituted by mushroom flour (Botella-Martínez et al., 2023). In this case, an increase in magnesium, iron and potassium was reported, but also a reduction in calcium content (Botella-Martínez et al., 2023). These modifications significantly enhance the nutritional profile of studied pork burgers, as long as the bioavailability of the minerals present in the meat are not compromised, and those incorporated are bioaccesible.

DTNC contributes TDF, mainly IDT, to pork burgers. Following Regulation (EC) No 1924/2006 BTN-6 and BTN-9 are sources of fibre and high fibre, since burgers contain at least 3 g of fibre per 100 g and 6 g of fibre per 100 g, respectively (Table 3). Other co-products used as non-meat ingredients in burger formulation have been reported to contribute fiber in the meat matrix, such as cocoa shells (Delgado-Ospina et al.,

Table 3

Chemical composition and mineral profile of raw and cooked pork burgers without DTNC (CB) and with the addition of DTNC.

	CB	BTN-3	BTN-6	BTN-9
Moisture (%)	65.26 ± 1.57^{a}	65.98 ± 1.52^{a}	65.94 ± 0.16^{a}	64.33 ± 0.62^{a}
Fat (%)	8.55 ± 0.05^{a}	8.09 ± 0.14^{b}	7.49 ± 0.09^{c}	7.10 ± 0.15^{d}
Protein (%)	17.62 ± 0.36^{a}	16.90 ± 0.25^{a}	17.11 ± 0.18^{a}	16.44 ± 0.97^{a}
Ash (%)	2.13 ± 0.12^{a}	1.91 ± 0.09^{a}	2.13 ± 0.09^{a}	2.23 ± 0.21^{a}
FDT (%)	-	2.08 ± 0.08^{c}	4.15 ± 0.16^{b}	6.23 ± 0.24^{a}
IDF (%)	-	1.98 ± 0.11^{c}	3.95 ± 0.22^{b}	5.93 ± 0.33^{a}
Na (mg/100g)	558.46 ± 33.91^{a}	489.46 ± 23.43^{a}	518.01 ± 14.66^{a}	529.50 ± 14.59^{a}
K (mg/100g)	343.98 ± 36.56^{a}	307.42 ± 16.15^{a}	320.99 ± 13.28^{a}	329.12 ± 1.99^{a}
P (mg/100g)	177.56 ± 21.01^{a}	159.14 ± 9.94^{a}	165.04 ± 7.98^{a}	170.50 ± 2.90^{a}
Mg (mg/100g)	22.29 ± 2.29^{b}	22.31 ± 0.07^{b}	24.98 ± 1.14^{ab}	27.90 ± 0.05^{a}
Ca (mg/100g)	9.00 ± 1.00^{b}	10.43 ± 1.51^{b}	12.06 ± 1.95^{b}	20.48 ± 1.44^{a}
Zn (mg/100g)	1.76 ± 0.30^{a}	1.60 ± 0.13^{a}	1.57 ± 0.08^{a}	1.81 ± 0.05^{a}
Fe (mg/100g)	0.95 ± 0.06^{a}	0.76 ± 0.01^{a}	0.82 ± 0.04^{a}	0.96 ± 0.11^{a}
Mn (μg/100g)	36.56 ± 2.20^{d}	51.46 ± 3.00^{c}	71.67 ± 2.17^{b}	89.73 ± 1.65^{a}
Cu (μg/100g)	28.10 ± 2.58^{b}	47.15 ± 10.07^{a}	46.63 ± 5.83^{a}	58.57 ± 4.68^{a}

DTNC: Supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9% of DTNC.

Different letters in the same row indicate statistically significant differences based on the Tuckey test ($p < 0.05$)

2022).

3.4. Physicochemical properties of pork burgers

Changes in meat color commonly indicate a loss of freshness and quality, primarily influenced by the redox balance of myoglobin. Discoloration of fresh meat denotes the dominance of oxidative processes. For example, in fresh pork meat, which contains low myoglobin, the color is pale greyish pink, whereas discoloration changes to color grey. The color appearance of meat and meat products are often used by consumers to purchase or reject it (El-Din Ahmed Bekhit et al., 2019; King et al., 2023). Incorporating non-meat ingredients into meat products is related to color modifications, limiting many times the amount to be incorporated or replaced. In the present study, the replacement of fat by DTNC in pork burger formulations contributed to significant changes in both color coordinates (L*, a* and b*) and chroma values (Table 4), which increased in a concentration-dependent manner ($p < 0.05$). These variations can be expected since DTNC has a hue color different to pork meat. Therefore, their incorporation modified color parameters. However, after the cooking process, only significant differences were observed in L* values among reformulated pork burgers with CB ($p < 0.05$). The pale brown color of DTNC mimics the tone of the brown color of burgers after cooking, making color differences less perceptible.

Concerning pH and water activity, these parameters were less affected by replacing fat with DTNC in burgers. For example, pH and water activity values were lower in BTN-9 than in CB ($p < 0.05$). However, although DTNC in high amounts promoted the reduction of pH and water activity, both values continued into the range of typical values for meat products.

3.5. Texture profile analysis (TPA) of pork burgers

Texture is a determinant attribute in meat and meat products. Final acceptance or repeat purchase of meat products is often related to texture properties. The impact on the texture of non-meat ingredients seems to be intrinsic to the ingredient used and to the amount employed For example, mushroom flour promoted a reduction in hardness and maintained cohesiveness (Botella-Martínez et al., 2023), while cocoa shell powder increased hardness and decreased cohesiveness (Delgado-Ospina et al., 2022) or legumes (pea, chickpea or lentil) reduced both, hardness and cohesion in pork burgers (Chandler and McSweeney, 2022). Other vegetable ingredients added to beef burgers such as lyophilized sweet cherries did not modify TPA results (Martín-Mateos et al., 2022). A recent study pointed out that the amount of meat (beef) is responsible for final texture properties (Souppez et al., 2025).

The presence of DTNC in pork burgers affected the hardness of burgers ($p < 0.05$). Both in raw and cooked burgers the hardness increased in a concentration-dependent manner ($p < 0.05$) (Table 4). The springiness values also increased with DTNC ($p < 0.05$), but only in raw burgers. Other secondary textural parameters such as gumminess and chewiness were also raised in a concentration-dependent manner. These results can be expected since both parameters are calculated using hardness values: gumminess = Hardness × Cohesiveness and Chewiness = Hardness × Cohesiveness × Springiness.

3.6. Lipid oxidation of pork burgers

The levels of lipid oxidation in raw pork burgers ranged from 0.23-0.49 mg MDA/kg, while in cooked pork burgers, the range was 0.67-0.91 mg MDA/kg (Fig. 2). DTNC exhibited antioxidant activity on raw burgers when added at 6 and 9 % ($p<0.05$). However, the antioxidant effect was not observed after the cooking process ($p>0.05$). Other co-products employed to reformulate burgers, such as pitahaya (*Hylocereus ocamponis*) peel flour and tomato peel flour, protected lipids from oxidation of both raw and cooked burgers (Karslıoğlu et al., 2024; Reyes-García et al., 2024). The presence of antioxidants, like flavonoids or carotenoids are related to this antioxidant activity. In the case of DTNC, the processing involved in making the tiger nut beverage and SFE has led to the removal of soluble antioxidants. Consequently, the lower lipid oxidation observed in the raw BTN-6 and BTN-9 formulations may be attributed to their reduced fat content compared to the control formulation. Additionally, DTNC may serve as a physical barrier in raw burgers. In none of the burgers, neither raw nor cooked, the TBA values detected exceed the limit for sensory detection of rancidity (>1.5 mg MDA/kg) (Domínguez et al., 2019).

3.7. Cooking properties of pork burgers

The cooking quality of burgers is related to cooking loss and size modifications (shrinkage and thickness) after their cooking because of several events such as protein denaturation y/o aggregation, water evaporation, fat melting and other chemical reactions. Consumers associate a low quality of meat products when these lose high amounts of fluids (water and fats) during the cooking process, and the size of burgers is exaggeratedly reduced because they are viewed as an indicator of poor meat quality and/or the effect of hormone treatments (Barbera and Tassone, 2006).

Table 4

Physicochemical properties and Texture profile analysis (TPA) of raw and cooked burgers without DTNC (CB) and with the addition of DTNC.DTNC: supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9% of DTNC.

	Raw				Cooked			
	CB	BTN-3	BTN-6	BTN-9	CB	BTN-3	BTN-6	BTN-9
L*	44.28 ± 4.87^{b}	48.27 ± 3.72^{ab}	49.19 ± 3.66^{ab}	50.22 ± 4.38^{a}	49.24 ± 3.78^{a}	45.26 ± 4.57^{ab}	43.96 ± 6.15^{b}	46.90 ± 5.33^{ab}
a*	2.79 ± 1.93^{cb}	3.72 ± 1.07^{b}	4.39 ± 1.11^{ab}	4.98 ± 1.00^{a}	5.24 ± 1.80^{a}	6.33 ± 2.73^{a}	6.91 ± 2.92^{a}	6.05 ± 2.91^{a}
b*	9.08 ± 2.08^{c}	11.45 ± 2.84^{b}	12.69 ± 2.12^{ab}	14.01 ± 1.73^{a}	16.51 ± 2.80^{a}	16.31 ± 2.14^{a}	16.37 ± 2.43^{a}	17.58 ± 2.40^{a}
C*	9.65 ± 2.27^{c}	12.10 ± 2.77^{b}	13.47 ± 2.12^{ab}	14.90 ± 1.75^{a}	17.39 ± 2.96^{a}	17.67 ± 2.42^{a}	18.04 ± 2.16^{a}	18.80 ± 2.57^{a}
ΔE^{*}	-	5.88 ± 3.26^{b}	6.98 ± 3.13^{b}	8.82 ± 3.10^{a}	-	5.96 ± 3.80^{a}	7.73 ± 4.53^{a}	5.66 ± 4.22^{a}
h*	73.88 ± 10.79^{a}	71.32 ± 6.27^{a}	70.75 ± 4.88^{a}	70.39 ± 3.74^{a}	72.53 ± 4.97^{a}	69.22 ± 8.14^{a}	67.03 ± 10.20^{a}	71.27 ± 8.64^{a}
pH	5.39 ± 0.03^{a}	5.41 ± 0.03^{a}	5.38 ± 0.03^{a}	5.30 ± 0.03^{b}	5.80 ± 0.03^{a}	5.72 ± 0.01^{ab}	5.69 ± 0.09^{b}	5.69 ± 0.04^{b}
aw	0.960 ± 0.001^{ab}	0.961 ± 0.001^{a}	0.959 ± 0.002^{ab}	0.957 ± 0.000^{b}	-	-	-	-
Hardness (N)	16.28 ± 2.51^{d}	29.07 ± 2.60^{c}	42.88 ± 8.98^{b}	69.16 ± 7.55^{a}	87.57 ± 8.09^{d}	135.63 ± 17.92^{c}	181.69 ± 15.61^{b}	230.50 ± 16.54^{a}
Adhesiveness (N s)	-0.71 ± 0.46^{a}	-0.55 ± 0.42^{a}	-0.12 ± 0.03^{a}	-0.10 ± 0.02^{a}	-	-	-	-
Springiness (mm)	0.18 ± 0.01^{b}	0.18 ± 0.01^{b}	0.20 ± 0.01^{ab}	0.22 ± 0.02^{a}	0.37 ± 0.02^{a}	0.38 ± 0.02^{a}	0.40 ± 0.01^{a}	0.39 ± 0.04^{a}
Cohesiveness	0.34 ± 0.03^{a}	0.32 ± 0.03^{a}	0.34 ± 0.02^{a}	0.37 ± 0.03^{a}	0.46 ± 0.03^{b}	0.48 ± 0.03^{b}	0.49 ± 0.03^{ab}	0.55 ± 0.03^{a}
Gumminess (N mm)	5.53 ± 1.16^{c}	9.31 ± 0.34^{c}	14.49 ± 2.52^{b}	25.56 ± 2.50^{a}	40.58 ± 5.70^{d}	65.01 ± 12.32^{c}	87.85 ± 5.51^{b}	126.01 ± 12.07^{a}
Chewiness	1.00 ± 0.21^{c}	1.71 ± 0.09^{c}	2.86 ± 0.51^{b}	5.58 ± 0.82^{a}	15.01 ± 2.37^{d}	24.44 ± 3.88^{c}	35.28 ± 2.03^{b}	48.53 ± 5.54^{a}
Resilience	0.11 ± 0.01^{ab}	0.09 ± 0.01^{b}	0.11 ± 0.01^{ab}	0.12 ± 0.01^{a}	0.16 ± 0.02^{b}	0.16 ± 0.02^{b}	0.17 ± 0.01^{b}	0.21 ± 0.02^{a}

DTNC: Supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9% of DTNC. Different letters in the same row indicate statistically significant differences based on the Tuckey test ($p < 0.05$)

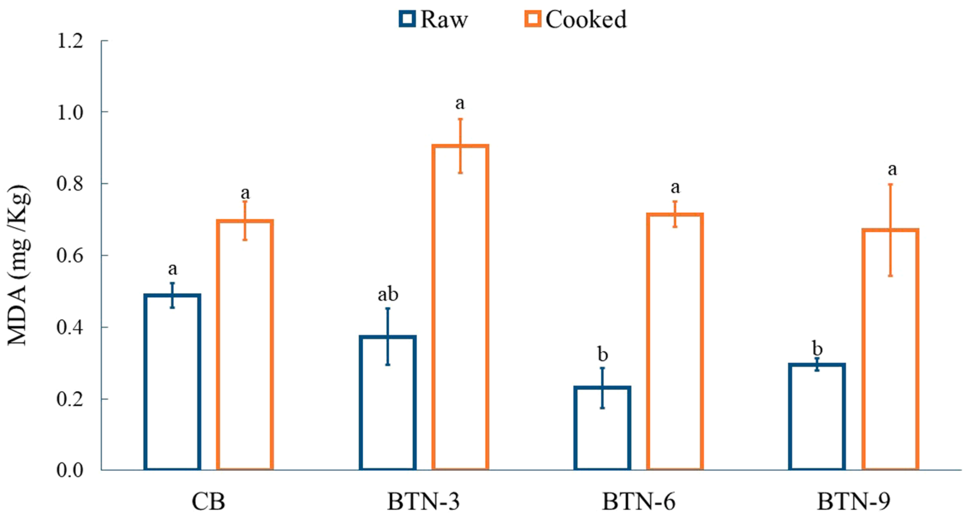

Fig. 2. Lipid Oxidation of raw and cooked pork burgers without DTNC (CB) and with addition of DTNC. DTNC: supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9 % of DTNC. Different letters indicate statistically significant differences based on the Tuckey test ($p < 0.05$)

The highest percentage of cooking loss was reported for CB, followed by BTN-3, BTN-6 and BTN-9 ($p < 0.05$) (Fig. 3). These results would indicate that the presence of DTNC reduces the loss of fluids, due to evaporation and leakage, during the cooking of burgers. It has been reported that the addition of plant-based ingredients to meat products decreased their cooking losses, which has been attributed to lower changes in the overall microstructure and fluid-holding properties in vegetables than in meat proteins by heating (Zhou et al., 2022). The results of the burgers' alteration in size (shrinkage and thickness) are shown in Fig. 3. The behavior of the burger shrinkage was the same as reported for cooking loss: the addition of DTNC decreased the shrinkage in a concentration-dependent manner ($p < 0.05$). Thickness increase showed a similar trend except for the BTN-3 sample. These variations in size and volume due to heating are mainly due to meat protein shrinkage. Therefore, the addition of non-meat ingredients is a way to reduce these negative effects in meat products. In this way, Föste et al. (2020) reported that ingredients with high insoluble fibers (such as DTNC), due to their hydration capacity, can improve food density, minimize shrinkage, retard staling, control moisture, and increase food stability.

3.8. *Microbiological quality of pork burgers*

The burgers employed for the sensory analysis reported an acceptable microbiological account (COMMISSION REGULATION (EC) No 1441/2007), with *Enterobacteriaceae* counts below 2.00 log and no presence of moulds or yeast. Additionally, the aerobic colony counts and lactic acid bacteria amounts were lower than 5 log/g (Supplemented Table 2)

3.9. *Sensory evaluation of pork burgers*

Sensory acceptance of reformulated products is essential both to guarantee the product's viability on the market and to continue research lines. Most of the 50 panelists who participated in the hedonist test consumed burgers once a week (38 %) or one at two weeks (20 %). All reformulated burgers (BTN-3, BTN-6 and BTN-9) scored lower than control burgers ($p<0.05$) in all the evaluated attributed (general aspect, color, hardness, juiciness, crumbliness, flavor and granularity), except general aspect and color where no significant differences were reported among CB and BTN-3 (Fig. 4). Furthermore, as shown in Fig. 4, the score of the attributes decreased as the amount of DTNC increased. All the sensory attributes in BTN-3 were scored higher than 5 (like slightly) and lower than 5 in BTN-9. However, in BTN-6 only granularity, crumbliness and juiciness were scored lower than 5, being these attributes the most affected by the DTNC addition. The perception of granularity relates to the particle size of the DTNC, enhancing this perception can be achieved by reducing the particle size and ensuring a more homogenous distribution. Crumbliness is related to the chewiness of the burgers (Table 4, cooked burgers), which was significantly increased by the addition of DTNC. The decrease in the perception of juiciness by the addition of DTNC could be related to the high WHC of this co-product, which has been also reported when other fibrous ingredients are added to meat products (Lucas-González et al., 2020).

The global acceptance of burgers was as follows: Control (7.68 ± 1.21) > BTN-3 (6.70 ± 1.51) > BTN-6 (5.10 ± 1.96) and BTN-9 (3.74 ± 1.96) ($p < 0.05$). Therefore, except for BTN-9, the rest of the studied formulation was acceptable by the 50 panelists.

4. Conclusions

The supercritical CO_2 extraction of oil from "horchata" co-products generates another co-product rich in insoluble fiber, protein and minerals. Supercritical-CO_2-defatted tiger nut milk co-products can be used in the meat industry as a fat substitute or to enrich meat products with fiber and minerals, especially manganese, calcium and copper. Furthermore, supercritical-CO_2-defatted tiger nut milk co-products prevent lipid oxidation in raw pork burgers and cooking loss and shrinkage after the cooking process. Nevertheless, negative effects on textural properties were observed, particularly in hardness and chewiness, which lowered the overall acceptance of cooked pork burgers. In conclusion, an effective valorization of "horchata" beverage co-products can be carried out using low amounts of supercritical-CO2-defatted tiger nut milk co-products to develop meat products, such as burgers. However, more research can be developed to understand the digestive behavior of the incorporation of non-meat ingredients into meat products on protein digestibility and mineral accessibility.

Ethical statement

Before starting the sensory analyses, each subject was informed about the specific characteristics of the product to be tasted and about what the analysis would consist of, and a written informed consent was signed by the participants. This project was approved by the Responsible

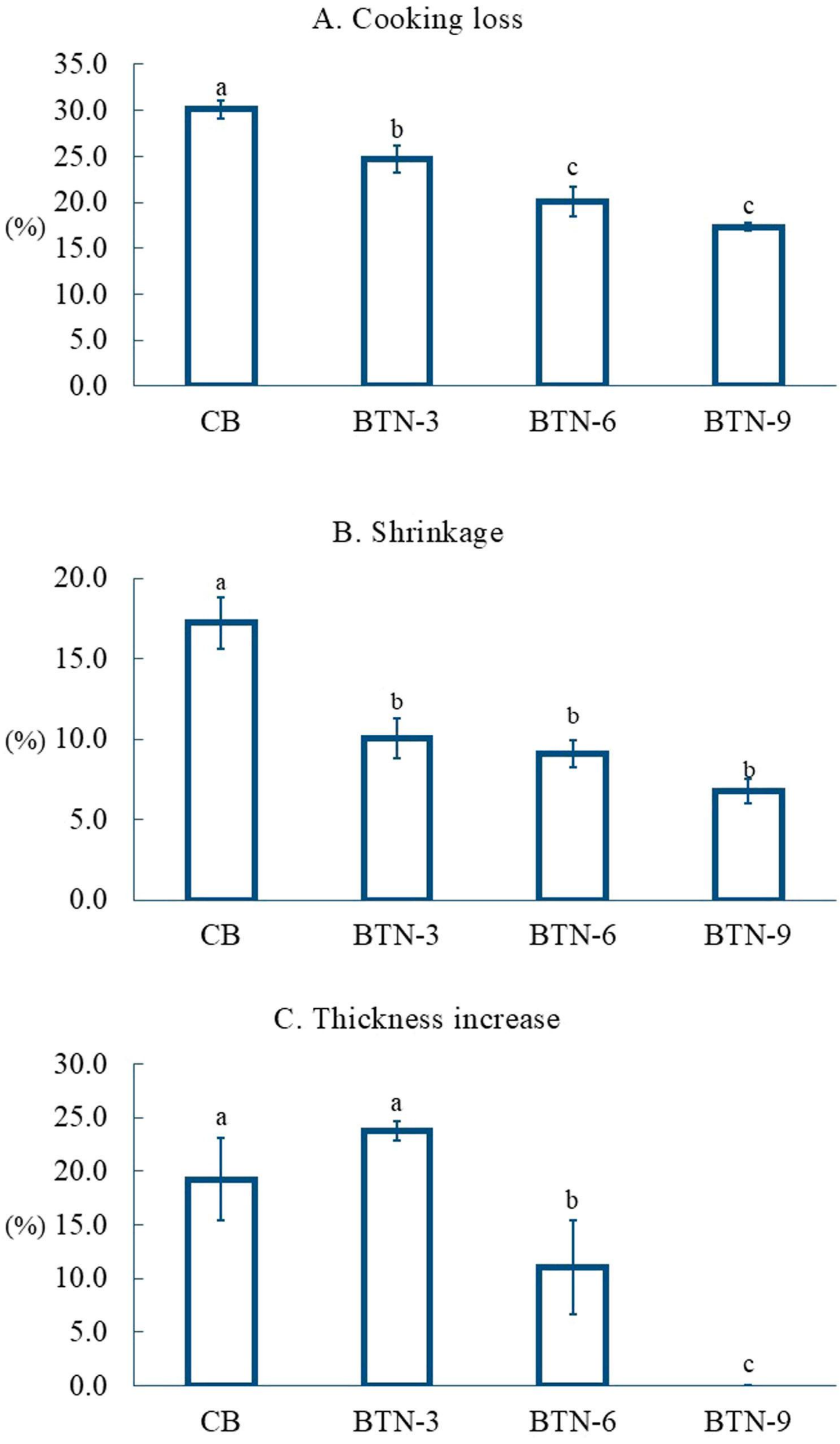

Fig. 3. Cooking quality of pork burgers without DTNC (CB) and with addition of DTNC. DTNC: supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9 % of DTNC. Different letters indicate statistically significant differences based on the Tuckey test ($p < 0.05$)

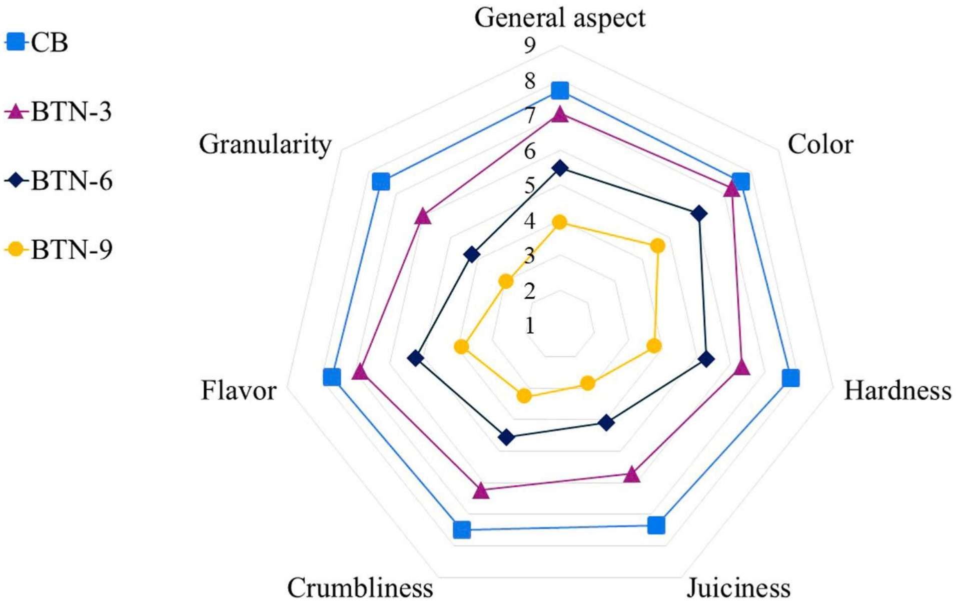

Fig. 4. Sensory evaluation of pork burgers without DTNC (CB) and with addition of DTNC. DTNC: supercritical-CO_2-defatted tiger nut milk co-product; CB: Control burger; BTN-3: burgers with 3 % of DTNC; BTN-6: burgers with 6 % of DTNC; BTN-9: burgers with 9 % of DTNC.

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CRediT authorship contribution statement

Raquel Lucas-González: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carmen Botella-Martínez:** Formal analysis, Conceptualization. **Manuel Salgado-Ramos:** Writing – original draft, Methodology, Investigation, Formal analysis. **Noelia Pallarés:** Writing – review & editing, Investigation. **Pedro V. Martínez-Culebras:** Writing – review & editing, Investigation. **Francisco J. Barba:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Juana Fernández-López:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization.

Declaration of competing 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100542.

Data availability

Data will be made available on request.

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