Impact of incorporation different levels of tritordeum flour on the nutritional, quality and sensory properties of beef burger samples

Rehab F. M. Ali[1]*, Norah A. Alabdullatif[1]

[1] Department of Food Science and Human Nutrition, College of Agriculture and Food, Qassim University, Buraydah 51452, Saudi Arabia

*Corresponding Author: Rehab F. M. Ali E-mail: reh.ali@qu.edu.sa , malkelanany@gmail.com

Summary

The current study aimed to investigate the impact of incorporating different levels of tritordeum flour (HTWF) on the nutritional, quality, and sensory aspects of beef burger samples. HTWF exhibits a unique nutritional profile with 64.55% carbohydrates, 17.36% crude protein, 13.2% crude fiber, and 2.97% ash. HTWF has remarkable functional features, including water absorption capacity (WAC, 169 g/100 g), oil absorption capacity (OAC, 140 g/100 g). The substitution of beef fat with HTWF resulted in considerable decreases in cholesterol content, significant enchantments nutritional profile of burger samples, particularly in terms of protein, fiber and ash content p≤ 0.05). The incorporation of HTWF into beef burgers has been shown to significantly improve cooking yields, moisture retention, and fat retention, while also enhancing the nutritional profile with increased phenolic compounds, antioxidant activity, carotenoids, and lutein content. (p≤ 0.05). The incorporation of HTWF significantly (p≤ 0.05) improves the overall acceptability of beef burgers.

Keywords Burger, Tritordeum flour, low fat, diet, antioxidants, lutein

Introduction

Beef burgers are popular for their taste and convenience, yet health concerns regarding processed meats are prompting consumers to seek healthier alternatives. Recent studies indicate that while traditional beef products are high in saturated fats, innovative approaches are being explored to enhance their health benefits and sensory qualities. Maki et al. 2021) **;** Anany et al., 2024-El**).**

In this regard, tritordeum, a hybrid of durum wheat and wild barley, offers significant agronomic and nutritional advantages, particularly for those seeking to reduce gluten intake. Its unique gluten composition results in a substantial reduction of immunogenic gluten peptides, making it a safer alternative for non-celiac gluten-sensitive individuals. Additionally, tritordeum flour is rich in beneficial nutrients, including carotenoids like lutein, fiber, and protein, which contribute to its health-promoting properties (Sánchez-León et al., 2021).

The incorporation of functional ingredients, such as tritordeum flour, into meat products like burgers presents a promising opportunity to enhance their nutritional profiles. However, this reformulation must carefully balance health benefits with sensory qualities to ensure consumer acceptance (El-Anany et al., 2024). Therefore, the main objective of the current investigation is to evaluate the impact of incorporating different levels of HTWF on the nutritional, quality, and sensory properties of beef burger samples.

Materials and Methods

Tritordeum flour (*Triticum* x *Hordeum*) **one package (10 Kg)** was obtained from Molino S. Giuseppe ZAPPELLA company, Mascalucia (Catania) Italy. Fresh lean beef and kidney fat were sourced from the fresh meat section of Tamimi markets in Buraidah, Qassim, Saudi Arabia. All chemicals and solvents were categorized as analytical.

Hydrated tritordeum whole meal flour (HTWF)

The process involves mixing 33.33% tritordeum whole meal flour and 66.66% cold water for (4 ^{o}C) The mixture is allowed to rest at controlled room temperature 25ºC for 24 hours, which aids in hydration and improves the flour's functional properties (El-Adawy et al., 2003).

Beef burger Formulations

Ten Kg of lean meat and 1.500 g of beef fat were individually ground using an 8 mm plate by a kitchen mincer (Panasonic Meat Grinder 1700 W, MK-GX1710KTZ, Japan). The meat was mixed with 5% spices mixture (each 100g of spices contains 40 g salt, 10 g garlic powder,10 onion powder, 10 cumin powder, 20 g black pepper, 4 g tripolyphosphate, 6 g ascorbic acid.) and 10 % flake ice. All burger samples were made with 65% lean beef. Consequently, the control burger was made with 65% lean meat and 20% beef fat.

The study aimed to substitute the kidney fat of the developed beef burger (20%) with five different levels of HTWF as follow:

T2.5) beef fat (17.5 g), HTWF (2.5 g)

T5.0) beef fat (15 g), HTWF (5 g).

T7.5), beef fat (12.5 g), HTWF (7.5 g)

T10) beef fat (10 g), HTWF (10 g)

T12.5 beef fat (7.5 g), HTWF (12.5 g)

The mixture was mixed by a wooden spoon, ground in a meat grinder (Univex MG22, USA) with a 0.5 cm plate, and formed into 100 g burgers (10 cm in diameter, 10 mm thick) using a burger press machine (FIMAR F10, Patty Press Italy). Beef burgers were put on plastic foam plates, covered with 10-micron polyethylene film, and stored in a freezer at -20 °C until until further analysis. The raw burger samples were cooked by using electric grill (Philips Contact Grill 5000 Series - 2200W), for 5 min on each side to ensure that the internal temperature of 75±1°C measured at the center of beef patty using a digital food thermometer (DOQAUS Food Thermometer, Instant Read Meat Thermometer).

Analytical methods

Proximate composition and Techno-functional properties

Proximate analysis which includes moisture, ash, fat, protein, crude fiber and carbohydrate contents. Were conducted using standard procedure of AOAC, 2012, AOAC 925.09B, AOAC method no. 930.22; AOAC 950.36; AOAC method no. 950.36; AOAC method no. 950.37). The carbohydrate content was computed by subtracting the sum of all proximal components from 100% (El-Anany et al., 2024). The energy value (kcal/100 g) was calculated with the Atwater conversion factor (Osborne and Voogt, 1978). Energy (kcal/100g) equals [9 × Lipids% + 4 × Proteins% + 4 × Carbohydrates%]. The absorption capabilities of water and oil were determined using the methodologies given by (Chandra et al.,2015).

Cholesterol assays

The cholesterol concentration (mg/100 g, dry weight basis) was assessed using the spectrophotometric procedure as described by Ramadhan et al. (2012). Petroleum ether was used to extract fats from the samples. The extracted fat underwent saponification using aqueous ethanolic KOH. The mixture solution was allowed to cool at ambient temperature, and 10 mL of petroleum ether was added. After cooling, acetic acid saturated with ferrous sulfate and concentrated sulfuric acid are added to develop the chromophore. The absorbance was measured at 490 nm against the reagent blank.

Total phenolic content, total flavonoid content and DPPH radical scavenging activity%

The assessment of total phenolic content, total flavonoid content, and DPPH radical scavenging activity were assessed using the procedures described by (Ali and El-Anany, 2025).

Total lutein and total carotenoids

Lutein extraction

Lutein extraction was conducted using (Murillo et al., 2010) procedures. The absorbance was measured at 445nm, and the lutein concentration was calculated using the following equation.

Concentration of lutein (µg/g of sample) =

A × V× dilution factor / E 1% cm × w Kamalambigeswari and Jeyanthi, 2016.

Where, A =Absorbance, V = Volume (ml), E 1% cm = Extinction coefficient of solvents and W= Weight in (g)

Total Carotenoids

Total carotenoids (mg/g) were determined using the procedures provided by (Abdel-Moatamed et al., 2024).
Total carotenoids (mg/g) = (1000 × A470) - [(2.860× Ca) - (129 × Cb)]/245. Ca represents chlorophyll a concentration (mg/g) at 666nm, while Cb represents chlorophyll b concentration (mg/g) at 653 nm.

Determination of cooking properties

The determination of cooking properties, including cooking yield moisture, and fat retention of burgers, was estimated according to the procedures described by (Yüncü *et al* 2021).

Colour measurement

The colour was measured at room temperature individually using a Hunter Lab Scanmodel colorimeter (Minolta cR-300, Minolta Corp., Ramsey, NJ) according the procedures described by (Bakhsh et al., 2021). The instrument was equipped with a standard D65 illuminant. Prior to use, the instrument was calibrated. For calibration, three common color slices were utilized: L* for lightness/darkness, b* for yellow+blue, and a* for redness values. Prior to the measurement, each sample was put into a transparent Petri dish. Carefully, it was observed that there were no spaces between the petri dish lids; in every measurement, the filled sample and the colorimeter's lens completed touch with the petri dish lid.

Sensory evaluation of cooked burger samples

Sensory evaluation of beef burger samples was conducted, as authorized by the Qassim University Ethics Committee (Committee of Research Ethics, Institutional Review Board, IRB), involved a structured methodology to ensure reliable results. A diverse panel of 45 healthy judges (25 females and 20 men, aged 19–45 years) from the staff and students at the College of Agriculture and Food, Qassim University, Saudi Arabia, was randomly selected, Panelists were informed about the burger components. The sensory properties evaluated included appearance, color, texture, juiciness, taste, odor, and overall acceptability, using a 9-point scale. To mitigate flavor carryover, panelists were provided with plain water between samples. Evaluations were performed in separate booths with white fluorescent lighting and a room temperature of 25°C).

Statistical analysis

The statistical analysis of data (five replicates (n = 5 replicates), (except sensory results (n = 45) was statistically examined in a randomized design using Excel (Microsoft Office 2007; Microsoft Corporation, Redmond, WA, USA) and SPSS version 18.0. Mean values and standard deviation were used to describe the results. Duncan multiple range tests were performed at the 0.05 level,

Results and Discussions

Proximate composition of tritordeum flour

Table 2 shows the proximate composition of tritordeum flour. The proximate composition of tritordeum flour reveals its significant nutritional advantages, making it an appealing ingredient for health-oriented food products. With a moisture content of 11.21%. Its low-fat content (1.92%) is beneficial for health-conscious consumers, while the high crude protein (17.36%) and crude fiber (13.2%) levels enhance its nutritional profile. Additionally, the carbohydrate content (64.55%) and ash content (2.97%) indicate a rich mineral profile, The energy content of tritordeum flour was 388.6 Kcal/100g. These findings align with the results presented by Suchowilska et al., (2021).

Techno-functional properties of tritordeum flour

The water absorption capacity (WAC) of tritordeum flour, measured at 169 g of H_2O per 100 g, is primarily due to its hydrophilic components, such as carbohydrates and fibers, which enhance moisture retention during baking. Similarly, the oil absorption capacity (OAC) of 140 g oil per 100 g flour plays a crucial role in flavor and moisture retention, thereby improving the sensory qualities of baked products (Li et al., 2023).

Proximate composition and cholesterol content of uncooked and cooked burgers partially substituted with different levels of HTWF

Table 3 shows proximate composition and cholesterol content of uncooked and cooked beef with different levels of HTWF.

The incorporation of HTWF into beef burgers significantly affects their moisture content, both in uncooked and cooked states. The addition of HTWF increases the moisture content due to its high WAC. This increase in moisture is proportional to the percentage of HTWF added, with higher substitutions leading to greater moisture retention. Substituting kidney fat with HTWF leads to a significant increase in protein content, with specific increases of 10.33%, 9.00%, and 5.72 % observed at 12.5%, 10%, and 7.5% substitution levels, respectively. The addition of HTWF enhances the protein levels in cooked burger samples, with increases of 11.03%, 9.82%, and 6.63% for 12.5%, 10%, and 7.5% supplementation, respectively. The highest fat content at 18.76% was recorded for control samples. Substituting beef fat with HTWF at levels of 12.5%, 10%, 7.5%, 5%, and 2.5% resulted in fat content reductions of 2.99, 2.29, 1.84, 1.50, and 1.12 times, respectively, compared to control samples. Similar studies have demonstrated that incorporating oat and chia, can reduce fat content of beef burgers (Saber et al., 2023). Control samples had the lowest ash content at 1.83%. Burgers with 12.5% HTWF showed the highest increase in ash content, reaching 2.54%. HTWF contains approximately 24% more ash than durum wheat, indicating its superior mineral composition (Suchowilska et al., 2021). The lowest crude fiber content 0.23% was recorded for control samples. Burgers with HTWF exhibited crude fiber content increases of 7.9, 6.5, 5.26, 3.86, and 2.86 times for 12.5%, 10%, 7.5%, 5.0%, and 2.5% substitutions, respectively. The incorporation of oat and chia flours into burger formulations significantly enhances their fiber content, leading to improved health benefits (Saber et al., 2023). The incorporation of HTWF into burger samples significantly ($p \leq 0.05$) influences their carbohydrate content, with the highest levels observed at a 12.5% substitution. Energy value of control sample was higher 1.07, 1.27, 1.40, 1.52, and 1.68 times than burgers substituted with 12.5, 25, 37.5, 50, and 62.5% of HTWF, respectively. The caloric content decreases as the percentage of HTWF increases.

Cholesterol content

Control sample recorded a cholesterol concentration of 129.60 mg/100 g. Addition of HTWF leadto significant decreases in cholesterol concentration, with reductions of 4.97, 6.86, 11.41, 14.12 and 20.9 % for 2.5, 5.0, 7.5, 10 and 12.5%substitutions, respectively (Table 3) Plant fibers, such as oat and chia flour, have been demonstrated to successfully minimize fat and cholesterol in burgers (Saber et al., 2023). Pijuayo pulp lowered fat content while simultaneously improving sensory properties, demonstrating that plant-based additives can improve both health and palatability (Liatas et al., 2024). The cholesterol concentration in cooked burger samples is significantly higherthan in uncooked samples, with increases ranging from 13.46% to 15.22% when expressed on a dryweight basis.

Effect of replacement of beef fat with HTWF on the cooking characteristics

Cooking yield

The control samples had the lowest yield (71.93%). Beef burgers with HTWF at levels of 2.5–12.5% exhibited cooking yields that were 1.01 to 1.10 times higher than those of the control samples. The incorporation of alternative flours has been shown to enhance the cooking performance of low-fat beef products by improving yields (El-Anany & Ali, 2018). This trend is supported by various studies that highlight the moisture retention and fat-binding properties of these adjuncts (El-Anany & Ali, 2018; Argel et al., 2020).

Moisture Retention

The lowest moisture retention 65.31 % was recorded for control samples. The highest moisture retention values 71.64 and 72.62 % were recorded for burger samples incorporated with 10 and 12.5 % HTWF, respectively (Table 4). meat ingredients-The use of noncan improve the moisture retention of burgers and enhanc e the tenderness and juiciness (Argel et al., 2020).

Fat Retention

Burgers with 5 - 12.5 % HTWF exhibited superior fat retention, outperforming both control samples and those with only 2.5% HTWF (Table 4). Specifically, burgers with 10% and 12.5% HTWF exhibited fat retention values that were 1.17 and 1.14 times higher than control samples. Themeat adjuncts-incorporation of non has been shown to significantly enhance theOAC of burgers (2020 ,.Argel et al).

Color parameters of uncooked and cooked burgers with different levels of HTWF

Table 5 shows color parameters of uncooked and cooked burgers with different levels of HTWF. The lowest Brightness (L*) value was recorded for control. The incorporation of HTWF into beef burgers significantly enhances their L* values compared to control. Similar findings were observed in other studies where the addition of wheat and sweet potato flour affected color attributes, demonstrating that flour blends can stabilize and improve color in meat products (Ogundipe et al., 2023). The highest a* value was recorded for control sample. Burger samples incorporated with different levels of HTWF had lower a* values compared to control. The observed decrease in a* values of burger samples containing HTWF, can be attributed to the interaction of myoglobin with various components in meat can lead to color changes, particularly when other ingredients are introduced (Faustman and Suman, 2017). Burger samples exhibited b* values from 18.03 to 23.66, with control samples showing the lowest values. The substitution of beef fat with HTWF in burger formulations significantly enhanced the (b*) values, these increases in b* values attributed to the high carotenoid and lutein content in HTWF.

Total phenolic content (TPC)

Control samples exhibited no TPC, while burgers with varying percentages of HTWF showed increasing TPC levels, with the highest at 16.05 mg GAE/g dry weight for the 12.5% HTWF sample. Significantly, TPC in cooked burgers was lower than in uncooked samples but still surpassed that of control samples.

Total flavonoids content (TFC)

Control samples showed no TFC, while burgers with varying percentages of HTWF exhibited increasing TFC levels, with the highest at 8.08 mg catechin equivalents (CE)/g dry weight for the 12.5% flour inclusion. This enhancement in TFC is attributed to the presence of flavonoids in tritordeum, which can counteract the thermal degradation typically observed during cooking.

DPPH radical scavenging activity

The DPPH radical scavenging activity observed in burger samples with varying concentrations of HTWF (12.5%, 10.0%, 7.5%, 5.0% and 2.5 %) indicates a significant antioxidant potential, with values of 8.79%, 7.10%, 5.31%, 3.54% and 1.76%, respectively. These findings align with other studies that emphasize the importance of natural antioxidants in food products, such as black garlic extract and date seed powder, which also demonstrated significant DPPH scavenging activities (Kelany et al., 2024).

Total carotenoids

The correlation between higher levels of HTWF and increased carotenoid content in burger samples indicates a significant enhancement in nutritional quality. Specifically, burger samples with 12.5%, 10.0%, and 7.5% HTWF showed total carotenoid concentrations of 0.17, 0.13, and 0.10 mg/g, respectively. This suggests that HTWF not only contributes to the color but also to the health benefits associated with carotenoids. (Table 6). Research indicates that tritordeum contains carotenoid levels that are 5 to 8 times higher than those found in durum wheat, making it a promising candidate for functional food development (Rodríguez-Suárez et al., 2014). Cooking process significantly reduces carotenoid content in burger samples due to various thermal and chemical reactions.

Total lutein

The analysis of lutein concentrations in burgers with varying percentages of HTWF reveals a significant correlation between HTWF content and lutein levels. Specifically, burgers containing 12.5%, 10.0%, and 7.5% HTWF exhibited lutein concentrations of 118.85, 100.90, and 60.35 µg/g, respectively, while control samples without HTWF showed no detectable lutein levels. (Table 6). The unique genetic makeup of tritordeum allows for a higher degree of lutein esterification, which may enhance its stability and bioavailability (Ávila et al., 2021). Cooking process lead to significant losses in lutein content in cooked burger samples.

Sensory evaluation of cooked beef burgers formulated with different levels of HTWF

Figure 1 shows sensory evaluation results of cooked burgers formulated with different levels of HTWF. Control sample had the lowest appearance score of 7.50. The highest scores were recorded for samples with 7.5%, 10.0%, and 12.5% HTWF. The ability of HTWF to retain moisture contributes to better visual and textural properties, enhancing the overall sensory profile of the burgers (Chin et al., 2024). Control samples received the lowest color score (8.00), while those with 10.0% and 12.5% flour achieved scores of 8.50 and 8.55, respectively. The addition of HTWF improved the color scores of the burgers. The addition of HTWF to beef burgers has been shown to enhance color scores, likely due to the presence of carotenoids and lutein, which contribute to the yellow hue. This improvement is significant when compared to control samples, which received the lowest color score. Carotenoids, including lutein, are known for their ability to impart a yellow color to food products, which can enhance visual appeal and consumer acceptance (Novello et al., 2014). The incorporation of HTWF in burger formulations has been shown to enhance texture scores, with values ranging from 8.00 for the control sample to 8.35 for those with 12.5% tritordeum flour. The addition of HTWF resulted in higher texture scores: 8.20, 8.25, and 8.35 for 7.5%, 10%, and 12.5% flour, respectively. J The lowest juiciness score 7.70 was recorded for control samples. The incorporation of HTWF into burger samples has demonstrated a significant impact on juiciness scores, with the highest ratings of 8.50, 8.55, and 8.55 recorded for samples containing 7.5%, 10%, and 12.5% HTWF, respectively. Control samples received relatively high taste ratings (8.10). However, the incorporation of HTWF into burgers has shown a significant impact on taste. Burgers with HTWF consistently outperformed control samples, suggesting a positive correlation between the flour's percentage and taste scores, peaking before a decline at 12.5% replacement (Fig 1). The highest odor score of 8.55 was achieved by control burgers and 2.5% HTWF. Conversely, higher percentages of HTWF (12.5%) resulted in lower odor scores. The highest overall acceptability scores were achieved with 5.0%, 7.5%, and 10% HTWF, attributed to its moisture retention, oil absorption, and appealing yellow color, which collectively improve consumer perception. The control sample scored the lowest at 7.97, highlighting the importance of ingredient selection in product development (Fig 1).

Conclusion

The incorporation of HTWF into burger formulations have been shown to significantly enhance the nutritional profile and alter the chemical composition of the products. Specifically, there are notable increases in moisture, protein, ash, fiber, and carbohydrates, alongside significant reductions in fat, cholesterol, and energy content. The addition of HTWF flour in burger samples significantly influences their color characteristics, particularly enhancing brightness and yellow hues while reducing redness. The incorporation of HTWF flour into burger mixtures has been shown to enhance their antioxidant properties. The highest overall acceptability scores were achieved with 5.0%, 7.5%, and 10% HTWF.

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Author contributions

R. F. M. A.: Conceptualization; methodology; software; validation; formal analysis; investigation; resources and data curation; writing; supervision; project administration; funding acquisition.

N. A. A.: Methodology; software; investigation; resources and data curation; funding acquisition.

Conflicts of Interest

No potential conflict of interest was reported by the author(s). In addition, the funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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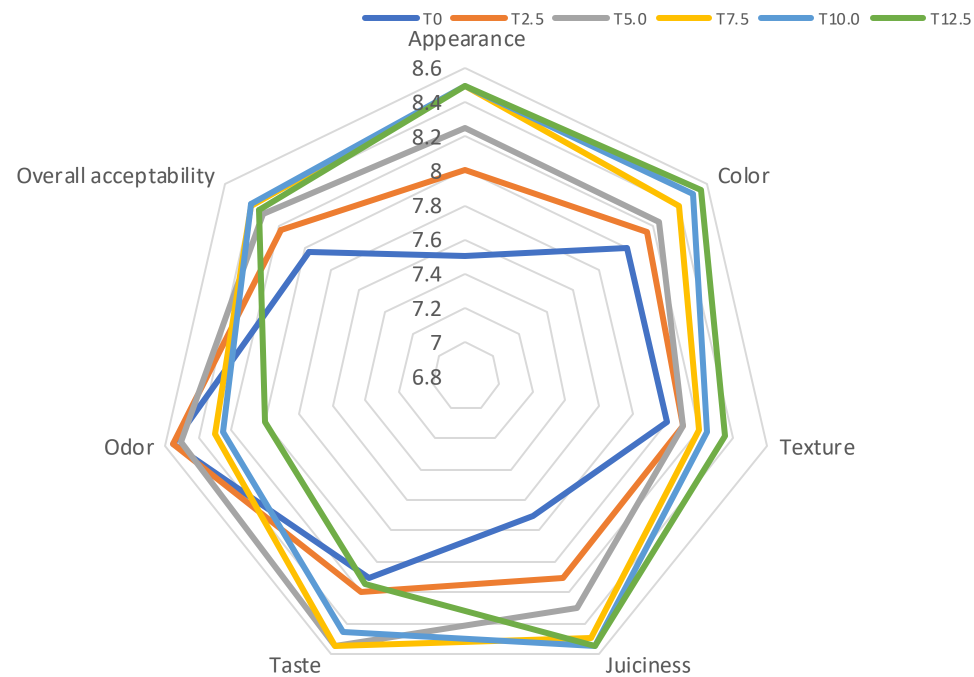
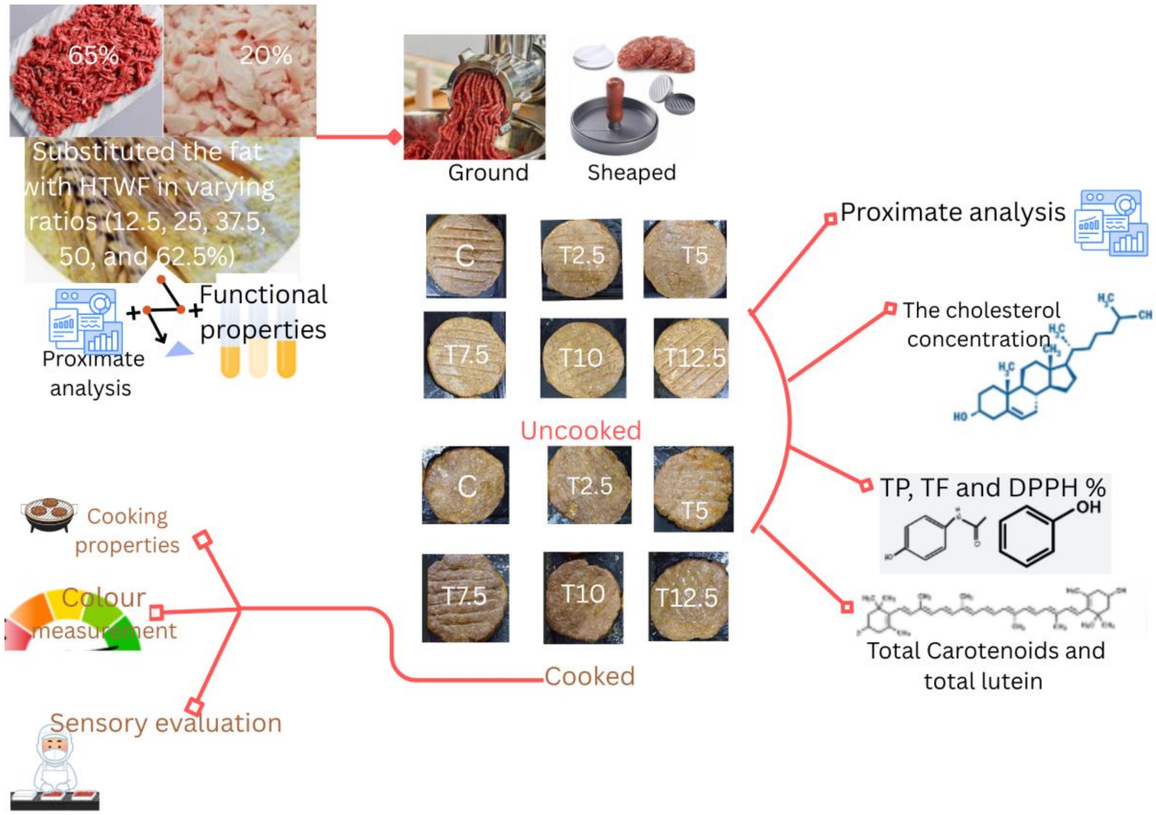


Figure 1 Sensory evaluation of cooked beef burgers formulated with different levels of hydrated tritordeum flour

T0 represents control beef burger sample. For treatments T1, T2, T3, T4 and T5 the beef kidney fat was substituted with HTWF at levels of 2.5, 5.0 ,7.5, 10.0 and 12.5 % respectively.

Impact of incorporation different levels of tritordeum flour on the nutritional, quality and sensory properties of beef burger samples.



Graphical abstract

Table 1 Beef burgers formulated with diffident levels of HTWF

Treatments	Lean beef (g)	Beef Kidney fat (g)	HTWF (g)	Flake ice (g)	Spices mixture**
T0	65	20.0	0.0	10	5
T2.5	65	17.5	2.5	10	5
T5.0	65	15.0	5.0	10	5
T7.5	65	12.5	7.5	10	5
T10	65	10	10	10	5
T12.5	65	7.5	12.5	10	5

*For treatments T2.5, T5.0, T7.5, T10 and T12.5 the kidney fat was replaced with HTWF at levels of 12.5, 25, 37.5, 50, and 62.5 % respectively.

**The mixture of spices contains 2 g salt, 0.5 g garlic powder, 0.5 onion powder, 0.5 cumin powder, 1 g black pepper, 0.2 g tripolyphosphate, 0.3 g ascorbic acid.

Table 2 Proximate composition and techno- functional properties of HTWF

Parameter	Tritordeum flour
Proximate composition (g per 100 g dry weight basis).	
Moisture (%)	11.21±0.72
Protein (%)	17.36±0.82
Fat (%)	1.92±0.11
Ash (%)	2.97±0.20
Crude fiber (%)	13.20±0.63
Carbohydrates (%)	64.55±1.87
Energy (kCal/100 g)	388.60±0.72
Techno-functional property	
WAC (g of H_2O/ 100g of sample)	169.00±2.50
OAC (ml of oil/ 100g of sample)	140.00±3.11

Values are means ± SD of five determinations

Table 3 Proximate composition and cholesterol content of uncooked and cooked burgers partially substituted with different levels of HTWF

	HTWF ratio (substitution %)					
	T0	**T2.5**	**T5.0**	**T7.5**	**T10.0**	**T12.5**
Uncooked						
Moisture content (%)	59.31^{e}±1.65	60.24^{d}±2.33	63.62^{c}±3.22	65.00^{b}±2.78	65.61^{ab}±3.01	66.61^{a}±2.78
Protein (%)	18.87^{f} ±0.99	19.10^{ef} ±1.25	19.47^{e} ±1.43	19.95^{e}±1.32	20.57^{d}±0.78	20.82^{d}±1.09
Fat (%)	18.76^{ab} ±1.02	16.74^{b} ±1.34	12.47^{cd}±1.01	10.17^{d} ±0.78	8.19^{e} ±0.76	6.27^{f} ±0.80
Ash	1.83^{h} ±0.82	2.17^{gh} ±0.98	2.35^{g} ±0.32	2.40^{fg} ±0.43	2.47^{f} ±0.08	2.54^{de} ±0.04
Crude fiber (%)	0.23^{g} ±0.03	0.66^{ef}±0.02	0.89^{e} ±0.03	1.21^{d} ±0.47	1.51^{cd} ±0.03	1.83^{bc} ±0.05
Carbohydrates (%)	1.00^{g} ±0.05	1.09^{fg} ±0.04	1.20^{f} ±0.03	1.27^{e} ±0.05	1.65^{bc} ±0.07	1.93^{ab} ±0.04
Energy value (kCal/100 g)	248.32^{b}±0.68	231.42^{bc}±0.89	194.91^{d}±0.80	176.41^{ef}±0.72	162.59^{g}±0.52	147.43^{h}±0.94
Cholesterol content (mg/100 g)	129.60^{de}±3.65	123.15^{f} ±2.54	120.70^{g}±2.22	114.80^{i}±3.13	111.30^{j}±2.76	102.45^{k}±2.74
Cooked						
Moisture content	53.88^{g} ±2.44	57.22^{f} ±2.32	57.92^{f} ±3.31	59.54^{e} ±3.42	60.14^{d} ±3.51	61.17^{cd}±3.81
Protein	22.29^{c}±1.54	22.38^{c} ±0.95	23.07^{b} ±1.54	23.77^{b}±1.52	24.48^{a} ±1.22	24.75^{a} ±1.90
Fat	19.62^{a} ±1.03	16.80^{b} ±0.98	13.70^{c}±0.94	10.42^{d} ±0.67	8.60^{e} ±0.95	6.72^{f} ±0.98
Ash	2.57^{de} ±0.89	2.65^{d} ±0.54	2.83^{c} ±0.03	2.94^{b} ±0.04	3.00^{a} ±0.02	3.08^{a}±0.65
Crude fiber	0.44^{f} ±0.01	0.70^{e} ±0.01	1.03^{de} ±0.05	1.68^{c} ±0.03	2.07^{b} ±0.01	2.27^{a} ±0.02
Carbohydrates (%)	1.20^{f} ±0.03	1.25^{e} ±0.02	1.45^{d} ±0.65	1.65^{c} ±0.05	1.70^{b} ±0.01	2.01^{a} ±0.04
Energy value (kCal/100 g)	270.54^{a}±0.88	245.72^{b}±0.98	221.38^{c}±1.20	195.46^{d}±1.60	182.12^{e}±1.10	167.52^{f}±1.43

Cholesterol content (mg/100 g)	148.11^{a} ±3.88	141.90^{b} ±4.95	136.95^{c} ±2.53	131.24^{d} ±2.51	127.65^{e} ±2.41	117.02^{h} ±3.83

T0 represents control beef burger sample. For treatments T1, T2, T3, T4 and T5 the beef kidney fat was substituted with HTWF at levels of 2.5, 5.0 ,7.5, 10.0 and 12.5 % respectively.

Values are means ± ± standard deviation of five determinations.

Means followed by the same letter across uncooked and cooked burgers are not significantly different ($p \leq 0.05$)."

Table 4. Effect of replacement of beef fat with various levels of tritordeum flour on the cooking characteristics of cooked beef burgers

Trait	Tritordeum flour addition ratio (substitution %)					
	T0	**T2.5**	**T5.0**	**T7.5**	**T10.0**	**T12.5**
Cooking yield (%)	71.93^{e}±2.33	72.01^{d}±2.77	75.21^{c}±3.00	76.43^{b}±2,56	78.21^{ab}±3.52	79.81^{a} ±2.49
Moisture retention (%)	65.31^{d} ±1.09	68.33^{c} ±2.59	68.44^{c} ±3.01	70.00^{b} ±33.66	71.64^{ab}±3.12	72.62^{a} ±3.11
Fat retention (%)	74.80^{f} ±2.58	72.72^{e} ±2.65	81.97^{d} ±3.95	77.94^{c} ±3.21	88.12^{b} ±3.87	85.39^{a} ±2.34

T0 represents control sample. For treatments T1, T2, T3, T4 and T5 the beef kidney fat was substituted with HTWF at levels of 2.5, 5.0 ,7.5, 10.0 and 12.5 % respectively. Values are means ± ± standard deviation of five determinations.

Means followed by the same letter are not significantly different ($p \leq 0.05$)."

Table 5. Color parameters (L*, a*, and b*) of uncooked and cooked beef burgers formulated with different levels of hydrated tritordeum flour

Trait	Tritordeum flour addition ratio (substitution %)					
	T0	**T2.5**	**T5.0**	**T7.5**	**T10.0**	**T12.5**
Uncooked						

Brightness (L*)	43.28^{e}±2.34	43.82^{d}±2.56	43.86^{d}±1.76	43.98^{c}±2.54	44.10^{b}±2.61	44.54^{a}±2.41
Redness (a*)	8.22^{a} ±0.98	8.05^{b} ±0.78	8.05^{b} ±0.45	7.56^{c} ±0.41	7.50^{d} ±0.56	7.28^{e} ±0.78
Yellowness (b*)	18.03^{h} ±.93	18.79^{g} ±0.94	18.98^{f} ±1.12	19.83^{e}±1.03	20.28^{b}±0.58	23.66^{a} ±0.98
Cooked						
Brightness (L*)	39.82^{i} ±2.45	40.76^{h} ±2.33	40.80^{gh}±1.46	40.92^{g} ±1.58	41.12^{ef}±1.88	41.13^{ef}±2.54
Redness (a*)	7.25^{e} ±0.78	7.15^{ef} ±0.12	7.10^{f} ±0.79	6.85^{g} ±0.35	6.80^{gh}±0.37	6.80^{h} ±0.44
Yellowness (b*)	20.48^{d} ±1.01	21.10^{c} ±0.89	21.13^{c} ±1.09	21.56^{bc}±0.67	21.90^{b} ±0.87	23.90^{a} ±0.98

T0 represents control beef burger sample. For treatments T1, T2, T3, T4 and T5 the beef kidney fat was substituted with HTWF at levels of 2.5, 5.0 ,7.5, 10.0 and 12.5 % respectively.

Values are means ± ± standard deviation of five determinations.

Means followed by the same letter across uncooked and cooked burgers are not significantly different ($p \leq 0.05$)."

Table 6. **Total phenolics, total flavonoids, DPPH radical scavenging activity %, total carotenoids, and total lutein of uncooked and cooked beef burgers with different levels of HTWF**

Trait	**Tritordeum flour addition ratio (substitution %)**					
	T0	**T2.5**	**T5.0**	**T7.5**	**T10.0**	**T12.5**
Uncooked						
Total phenolics (mg GAE/g dry weight	ND	3.45^{g}±0.21	6.11^{e}±0.89	9.41^{c}±0.97	12.96^{b}±0.93	16.05^{a}±0.66
Total flavonoid mg catechin equivalents (CE)/g dry weight	ND	1.97^{ef}±0.08	3.01^{d}±0.54	4.37^{c}±0.54	6.98^{b}±0.87	8.08^{a}±0.93
DPPH radical scavenging activity %	ND	1.76^{e}±0.07	3.54^{cd}±0.62	5.31^{bc}±0.38	7.10^{ab}±0.76	8.79^{a}±0.67
Total carotenoids (mg/g)	ND	ND	0.07^{d}±0.001	0.10^{c}±0.004	0.13^{b}±0.06	0.17^{a}±0.05

Total lutein (µg/g of sample)	ND	ND	27.04[d]±0.76	60.35[bc]±1.84	100.90[ab]±2.68	118.85[a]±2.87
Cooked						
Total phenolics (mg GAE/g dry weight	ND	2.41[h]±0.06	4.60[f]±0.65	7.29[d]±0.84	9.71[c]±0.74	12.14[b]±0.48
Total flavonoid mg catechin equivalents (CE)/g dry weight	ND	ND	2.02[e]±0.31	3.91[cd]±0.34	5.80[bc]±0.63	7.86[ab]±0.76
DPPH radical scavenging activity %	ND	ND	2.45[d]±0.13	3.78[c]±0.28	5.00[bc]±0.47	6.20[b]±0.42
Total carotenoids (mg/g)	ND	ND	ND	0.09[cd]±0.006	0.10[c]±0.003	0.12[b]±0.004
Total lutein (µg/g of sample)	ND	ND	ND	44.95[c]±1.95	70.88[b]±1.78	100.97[ab]±3.78

T0 represents control beef burger sample. For treatments T1, T2, T3, T4 and T5 the beef kidney fat was substituted with hydrated HTWF at levels of 2.5, 5.0 ,7.5, 10.0 and 12.5 % respectively.

Values are means ± ± standard deviation of five determinations.

Means followed by the same letter across uncooked and cooked burgers are not significantly different ($p \leq 0.05$)."