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RSST010 - COMPARATIVE ANALYSIS OF KETO BREAD FORMULATIONS: SENSORY, SHELF-LIFE, AND QUALITY ATTRIBUTES

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ABSTRACT

The rising popularity of ketogenic diets, particularly among individuals managing diabetes or pursuing weight loss, has driven demand for low-carbohydrate bread alternatives. However, substituting wheat flour with low-carbohydrate, high-fat flours can alter sensory qualities and affect consumer acceptance, and research on their quality and shelf-life remains limited. This study compared sensory, shelf-life, and quality attributes of keto bread made with almond, oat fiber, coconut and walnut flours, using standard wheat bread as a control. Shelf-life was assessed via microbial counts and pH over 12 days, while quality parameters included loaf volume, texture, moisture, and colour. Sensory evaluation using a 9-point hedonic scale involved 50 panellists. Almond flour bread showed the highest microbial stability, while oat fiber bread has the smallest pH variation and highest moisture content. Both almond and oat fiber breads had the highest absolute loaf volume, though wheat bread recorded the highest specific volume. Almond flour bread also had the softest, most springy crumb, wheareas coconut flour bread achieved highest overall preference among keto variants. These findings provide a scientific basis for optimising keto bread formulations with improved sensory quality and stability.

Keywords – ketogenic diet, low-carbohydrate, high-fat, keto bread

INTRODUCTION

Bread has been a dietary staple for thousands of years, with current average annual consumption estimated at 59-70 kg per person (Dong & Karboune, 2021, Mesta-Corral et al., 2024). Traditionally made from wheat flour, bread provides protein, complex carbohydrate, dietary fiber, vitamins, and minerals (Dong & Karboune, 2021). However, wheat bread is classified as a high-glycemic index (GI) food due to its fast-difesting polysaccharides, which can cause rapid spike in blood glucose levels (Mesta-Corral et al., 2024). The increasing popularity of ketogenic diet and gluten-free diets that is characterised by low carbohydrate, high fat, and moderate protein intake has created demand for alternative bread formulations (Gillespie & Ahlborn, 2021; Hopkin et al., 2022). Such diets are popular among individuals managing diabetes, seeking weight loss, or addressing neurological conditions like autism, Alzheimer's and parkinson's disease (Gillespie & Ahlborn, 2021). Since wheat flour is unsuitable for keto bread, low-carbohysrate flours derived from nuts, seeds, soy, and certain legumes are preferred (Gillespie & Ahlborn, 2021; Hopkin et al., 2022). Despite rising interest in ketogenic products, keto bread production faces sensory and textural challenges due to the absence of gluten, potentially reducing consumer acceptance (Gillespie & Ahlborn, 2021). Existing keto bread options are limited, with almond flour being the most common base, and little research exploring other low-carbohydrate, high-fat flours. There is a lack of scientific data on the shelf-life, colour, and other quality attributes of these breads despite colour is a key factor in consumer perception (Spence, 2018). This knowledge gap limits variety and innovation in the ketogenic bread market.

OBJECTIVE

This study aims to determine and compare the shelf-life and quality properties of keto bread prepared with different types of flours against conventional wheat bread. It also seeks to evaluate consumer acceptability of the various keto bread formulations through sensory analysis, assessing factors such as taste, texture, aroma, and overall preference. Furthermore, the research intends to identify the best keto bread formulation by integrating findings from microbial analysis, quality assessment, and sensory evaluation, ensuring an optimal balance of safety, quality, and consumer satisfaction.

METHODOLOGY

Four keto bread formulaions were prepared by using almond flour (AF), oat fiber flour (OF), coconut flour (CF), or walnut flour (WF), alongside a wheat flour as control sample (CTRL). Ingredients were weighed according to standardised recipes, mixed, proofed for 40 minutes and baked at 175℃ for 50 minutes for keto breads, and 22 minutes for control. Shelf-life analysis was conducted over 12 days at 3-day intervals, comprising of microbial analysis by using a hemocytometer, with bread suspensions diluted, stained with methylene blue, and counted microscopically. The shelf-life analysis also comprises of pH measurement by blending bread with distilled water to get 1:10 suspension, filtering, and recording pH with a calibrated pH meter. Next, quality attributes were measured 24 hours after baking, comprising of loaf volume analysis using the rapeseed displacement method to determine absolute and specific volume. Secondly, moisture analysis using the AACC two-stage bread moisture method. Thirdly, texture analysis was done by using a Texture Profile Analysis with a cylindrical probe. Lastly, colour analysis was done by using a calibrated chroma meter to get the data of L*, a* and b*. Sensory evaluation involved 50 untrained panelists at UiTM Kuala Pilah using a 9point hedonic scale to rate the appearance, aroma, texture, taste, and overall acceptability. Samples were coded with random three-digit numbers, and water was provided for palate cleansing. All data were analysed by using the IBM SPSS version 27. Two-way ANOVA was used for microbial and pH data, and one-way ANOVA for loaf volume, moisture, texture, colour, and sensory results, with significance at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1: Cells concentration of different keto breads and wheat bread for 12 days with 3 days interval.

Day	Concentration Of Cells (cells/mL)	Bread variation				
		AF	OF	CF	WF	CTRL
0		$2.18\times10^{7}\pm 2.55\times10^{6}{}_{Ab}$	$1.64\times10^{7}\pm1.70\times10^{7}{}_{ABb}$	$1.76\times10^{7}\pm2.26\times10^{6}{}_{Ab}$	$2.5\times10^{7}\pm5.37\times10^{6}{}_{Bb}$	$2.90\times10^{7}\pm2.83\times10^{5}{}_{Bb}$
3		$1.04\times10^{7}\pm 3.96\times10^{6}{}_{Ab}$	$1.74\times10^{7}\pm1.98\times10^{6}{}_{ABb}$	$2.16\times10^{7}\pm1.13\times10^{6}{}_{Ab}$	$2.22\times10^{7}\pm5.94\times10^{6}{}_{Bb}$	$3.80\times10^{7}\pm6.79\times10^{6}{}_{Bb}$
6		$1.06\times10^{9}\pm1.41\times10^{8}{}_{Aa}$	$1.40\times10^{9}\pm6.79\times10^{8}{}_{ABa}$	$1.22\times10^{9}\pm8.49\times10^{7}{}_{Aa}$	$8.20\times10^{8}\pm2.55\times10^{8}{}_{Ba}$	$7.20\times10^{8}\pm2.26\times10^{8}{}_{Ba}$
9		$8.60\times10^{8}\pm1.41\times10^{8}{}_{Aa}$	$1.06\times10^{9}\pm2.55\times10^{8}{}_{ABa}$	$1.14\times10^{9}\pm3.11\times10^{8}{}_{Aa}$	$1.06\times10^{9}\pm8.49\times10^{7}{}_{Ba}$	$1.18\times10^{9}\pm2.83\times10^{7}{}_{Ba}$
12		$6.80\times10^{8}\pm2.26\times10^{8}{}_{Aa}$	$7.00\times10^{8}\pm8.49\times10^{7}{}_{ABa}$	$1.86\times10^{9}\pm3.68\times10^{8}{}_{Aa}$	$8.80\times10^{8}\pm0.00^{Ba}$	$7.60\times10^{8}\pm5.66\times10^{7}{}_{Ba}$

Note: Upper case letters represent difference between bread variations and lower case letters represent differences of each variation at different day, Values with common letters are not significantly different ($p < 0.05$)

Microbial activity which is the analysis under shelf-life analysis, showed low initial microbial counts for all breads, indicating clean production. OF bread had the lowest count on day 0, likely due to the antibacterial properties of plant fibers (Zamora-Mendoza et al., 2022). Microbial growth increased over storage, with coconut (CF) bread reached the highest count by day 12, possibly from its higher moisture retention (Al-Zoghbi et al., 2025). AF bread exhibited the greatest microbial stability, consistent with the antimicrobial properties of almond skin extracts (Badalamenti et al., 2022).

Table 2: pH value of different keto bread formulations and control bread for 12 days with 3 days interval

Day	Bread variation				
	AF	OF	CF	WF	CTRL
0	8.26 ± 0.03^{Da}	9.04 ± 0.03^{Aa}	8.56 ± 0.02^{Ba}	8.63 ± 0.02^{Ca}	5.55 ± 0.02^{Ea}
3	7.63 ± 0.02^{Db}	8.89 ± 0.03^{Ab}	8.36 ± 0.02^{Bb}	7.95 ± 0.07^{Cb}	5.54 ± 0.01^{Eb}
6	7.08 ± 0.02^{Dc}	8.68 ± 0.01^{Ac}	8.03 ± 0.10^{Bc}	7.75 ± 0.06^{Cc}	5.48 ± 0.01^{Ec}
9	7.02 ± 0.04^{Dd}	8.53 ± 0.03^{Ad}	7.36 ± 0.04^{Bd}	7.63 ± 0.03^{Cd}	5.26 ± 0.02^{Ed}
12	6.95 ± 0.03^{De}	8.21 ± 0.03^{Ae}	7.34 ± 0.03^{Be}	7.43 ± 0.03^{Ce}	5.13 ± 0.01^{Ee}

Note: Upper case letters represent differences between bread variations and lower case letters represent differences of each variation at different day, values with common letters are not significantly different ($p < 0.05$)

pH values of all keto breads decreased during storage, likely due to microbial metabolism producing acidic by-products (Preetha & Narayanan, 2020). OF bread had the smallest pH change which is from 9.04 to 8.21, suggesting strong pH stability. Meanwhile, CTRL bread was consistently more acidic, providing a more favourable environment for spoilage organisms.

Table 3: Absolute volume and specific volume of different keto bread formulations and wheat bread

Bread variation	Absolute volume (cm^3)	Specific volume (cm^3/g)
AF	438.33±47.52^a	1.80±0.23^b
OF	438.33±16.07^a	1.76±0.09^b
CF	361.67±59.65^a	1.43±0.20^b
WF	370.00±0.00^a	1.46±0.20^b
CTRL	211.67±40.10^b	2.93±0.72^a

Note: Values with common letters are not significantly different ($p < 0.05$)

AF and OF breads recorded the highest absolute loaf volumes among keto breads, showing that these flours can support better gas retention during proofing. This may be due to almond flour's protein content aiding structure formation and oat fiber's ability to absorb water that improved dough viscosity. However, CTRL bread had the highest specific loaf volume, reflecting the structural advantage of gluten in trapping fermentation gases (Monteiro et al., 2021). CF and WF breads had lower volumes, possibly due to denser crumb structures from their high fiber and fat content, which can hinder gas expansion.

Table 4: Moisture of different keto bread formulations and wheat bread

			Bread variation		
Moisture (%)	AF	OF	CF	WF	CTRL
	49.47±0.75^a	51.82±1.14^a	49.06±1.73^a	50.12±0.88^a	35.53±2.45^b

Note: Values with common letters are not significantly different ($p < 0.05$)

OF bread had the highest moisture content that can be linked to the water-binding ability of its soluble and insoluble fibers. High moisture may improve perceived softness but it also can shorten shelf-life due to microbial susceptibility (Zambrano et al., 2019). AF and WF breads showed moderate moisture levels, while CF bread had relatively low moisture, which may lead to its denser texture but also prolong its shelf-life if water activity is reduced.

Table 5: Texture attributes of different keto bread formulations and wheat bread

Bread variation	Firmness (g)	Springiness (%)
AF	295.95±45.70^c	57.51±0.27^a
OF	723.11±28.14^b	51.51±2.25^b
CF	1966.95±168.74^a	52.04±1.25^b
WF	1819.62±116.25^a	52.84±1.70^b
CTRL	737.62±61.75^b	54.29±2.13^{ab}

Note: Values with common letters are not significantly different ($p < 0.05$)

CF bread had the highest firmness, indicating a dense and hard crumb, while AF bread was the softest, likely due to its high fat content that promotes softer texture (Barreca et al., 2020; Hao et al., 2014). AF bread was also less firm than OF bread, consistent with the finding that oat fiber's strong water-binding ability contributes to greater firmness (Gillespie & Ahlborn, 2021). Among keto breads, OF had the most similar firmness to wheat bread (CTRL). In terms of springiness, AF bread had the highest value and differed significantly from other keto breads but not from CTRL. Since keto breads lack gluten, their springiness is derived from high fat, protein, and fiber content that help to maintain resilience and texture over time (Gillespie & Ahlborn, 2021).

Table 6: Value of L*, a* and b* for crumb of different keto bread formulations and wheat bread

	Colour of crumb		
Bread variation	**L***	**a***	**b***
AF	43.65±4.12^{b}	2.15±0.02^{b}	19.04±1.79^{a}
OF	42.77±2.84^{b}	2.61±0.29^{b}	19.04±1.09^{a}
CF	47.19±0.60^{b}	2.06±0.2^{b}	18.80±0.09^{a}
WF	42.04±4.86^{b}	5.50±0.32^{a}	13.57±1.53^{b}
CTRL	57.54±1.97^{a}	-2.81±0.05^{c}	16.00±0.68^{ab}

Note: Values with common letters are not significantly different ($p < 0.05$)

Table 7: Values of L*, a* and b* for crust of different keto bread formulations and wheat bread

	Colour of crust		
Bread variation	**L***	**a***	**b***
AF	31.46±1.40^{ab}	9.84±0.48^{a}	16.24±0.94^{b}
OF	35.72±1.35^{a}	6.25±0.23^{c}	19.66±0.52^{a}
CF	24.33±0.75^{c}	7.64±0.64^{bc}	13.15±0.28^{c}
WF	28.97±2.79^{bc}	7.87±0.78^{b}	14.96±1.46^{bc}
CTRL	25.51±2.04^{c}	10.47±0.54^{a}	14.10±0.42^{bc}

Note: Values with common letters are not significantly different ($p < 0.05$)

Colour values (L*, a*, b*) varied significantly among formulations. AF and CF breads had lighter crumbs as indicated by the higher L* value, compared to WF and OF breads. The crust of AF bread exhibited higher browning with lower L* and higher b* value, potentially enhancing visual appeal. Colour differences are influenced by flour pigmentation, Maillard browning, and sugar content, with keto flours generally producing less intense browning than wheat flour due to lower sugar levels. (Spence, 2018).

Table 8: Sensory properties of keto bread

Attributes		Bread variation		
	AF	OF	CF	WF
Colour	6.72±1.51^{a}	6.88±1.15^{a}	6.90±1.31^{a}	5.80±1.88^{b}
Aroma	5.76±1.91^{a}	5.72±1.71^{a}	6.18±1.99^{a}	5.68±1.95^{a}
Taste	4.88±1.81^{ab}	4.62±1.84^{b}	5.70±2.02^{a}	5.68±2.18^{a}
Aftertaste	4.86±1.82^{bc}	4.44±1.88^{c}	5.86±1.91^{a}	5.68±2.01^{ab}
Eggy flavour	4.48±1.81^{b}	4.10±1.84^{b}	5.74±1.69^{a}	5.48±1.96^{a}
Overall acceptability	5.20±1.88^{ab}	4.82±1.98^{b}	6.16±1.86^{a}	5.82±1.98^{a}

Note: Values with common letters are not significantly different ($p < 0.05$)

Panellists rated CF bread the highest for colour, followed by OF bread, with WF bread was the lowest. This aligns with instrumental colour data, where CF bread had the highest crumb lightness (L*) and WF bread was the lowest, indicating a preference for lighter crumbs (Spence, 2015). Aroma scores showed no significant differences among keto breads, suggesting flour type did not significantly affect aroma. For taste and aftertaste, CF bread scored the highest and OF bread was the lowest. The cleaner aftertaste of CF bread may have contributed to its higher acceptance. Overall acceptability followed the same trend, where CF bread ranked the highest, OF bread was the lowest, with a significant difference between those two. CF bread's strong score across multiple attributes suggested that it was the most appealing keto bread to panellists, while OF bread was the least preferred.

CONCLUSION

This study compared the shelf-life, quality attributes, and sensory acceptance of four keto bread formulations with wheat bread. AF bread showed the best microbial stability, while OF bread maintained the most stable pH, indicating both as strong options for shelf-life. AF and OF breads achieved the highest absolute loaf volumes, with OF also having the highest moisture content. AF bread had the softest texture, and OF bread's firmness was closest to wheat bread. CF bread had the lightest crumb colour and was most preferred in sensory evaluation, scoring highest in taste, aftertaste, colour, and overall acceptability. Overall, each flour demonstrated specific strengths, but no single formulation excelled in all aspects. Future research could focus on combining these flours to develop a keto bread with balanced sensory appeal, structural quality, and extended shelf-life.

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