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Comparison of the polyamine content of white, whole wheat and rye bread

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ABSTRACT

Polyamines participate in many biological processes, predominantly in cell growth and proliferation. Body polyamine pool is provided through de novo biosynthesis, diet, and microbiota. However, data on polyamines in bread are limited. Therefore, we aimed to ascertain the polyamine levels of different bread species and whether it change after a time. Thirty bread samples of white, whole wheat, and rye bread were analysed using HPLC at day of procurement and after four days. Crumb/crust ratio was calculated to measure polyamine content in a single serving of bread. Total polyamine content increased in order of white, whole wheat, and rye bread in both crumb and crust. The polyamine level varied as follows: spermidine > spermine > putrescine, except for whole wheat. The difference in polyamine levels in the crumb and crust was significant. After four-day, the difference in total polyamine content of crumb was found significant. Total polyamine content (nmol) of a single serving of white, whole wheat and rye bread is 1170.32, 3496.72, and 3850.84 respectively. The polyamine content of bread varied according to both type and regions of the bread. Storage at 20-24°C led to elevation of the polyamine level

Keywords: polyamines, bread types, putrescine, spermidine, spermine

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INTRODUCTION

Polyamines (putrescine, spermidine and, spermine) are aliphatic polycationic molecules present in all cell types. They are involved in apoptosis, cell division and differentiation, cell proliferation, DNA and protein synthesis and gene expression[1]. The polyamine pool is derived from three primary sources: de novo synthesis, dietary intake, and microbiota[2], however, the diet provides much more polyamines than does de novo biosynthesis. As dietary polyamines are completely absorbed, diet might be an effective source[3]. Polyamines play a vital role in rapidly dividing cells such as immune system and digestive system. Since polyamines take part in cell proliferation, they are also involved in carcinogenesis. Therefore, studies investigate whether a diet low in polyamines has a beneficial effect on cancer[4-6]. Additionally, various pathological conditions, including inflammation, renal failure, stroke and diabetes are associated with the polyamine levels[7].

Polyamines found in both animal and vegetable are crucial exogenous sources. Since diet provides a larger quantity of polyamines than the endogenous biosynthesis, dietary polyamines are important for health. A diet of an adult provides a daily supply of several micromoles of polyamines. The effects of dietary polyamines might be detrimental, neutral or beneficial depending on individual's health condition. Increasing dietary polyamines is beneficial in rapid growth such as during the neonatal period, wound healing and after surgery while cancer patients are advised to reduce dietary polyamines for a better quality of life[8].

Grain-based foods supply much of the individuals' energy and nutrient requirements, providing 25-50% of energy in Western diets and over half of the energy intake of world population. Moreover, they are significant sources of carbohydrates, dietary fibre, micronutrients and plant-based protein[9]. Bread is a staple food and contributes at least 10% of energy requirements. Europeans consume on average 160 g per day (4-5 slices/day)[10]. As for Türkiye, it is estimated that 39.5% of daily energy intake is derived from bread and grains. On average, men consume 227 g of bread per day, while women consume 134 g[11]. It has been established that bread plays significant role in spermine intake, and the wheat products are identified as the primary source of spermidine in Türkiye[2].

In addition to macro- and micronutrients, we propose monitoring daily polyamine intake to evaluate nutritional status. Polyamine contents in foods vary widely between and even within food types due to origin, processing, storage conditions, seasonal variation and different methodological applications of foods[12]. In general, meat is rich in spermine, plant-based foods contain mostly putrescine and spermidine, dairy products include mainly putrescine and sper-

midine, and among them, cheese have higher polyamine values depending on fermentation conditions. The polyamine-rich foods consumption increases blood polyamine levels. The bread polyamine content is associated with flour source, fermentation and, baking conditions[12,13].

Although many studies have revealed the health effects of polyamines, the number of studies on the polyamine content of foods is limited. Furthermore, none of the studies have investigated the polyamine content of bread crumb and crust separately and how it changes after a storage period. It is acknowledged that dietary polyamines have significant impacts, and that the consumption of bread in the world is widespread. The objective of this study was to establish the polyamine concentration in the crust and crumb of three distinct bread types and to ascertain the alteration of polyamine level after four days of storage.

METHODOLOGY

General procedure

As seen at Figure 1, a total of ten samples of three kinds of bread were purchased from ten distinct bakeries. Then, crust and crumb were weighed in the laboratory. Crumb/crust ratio was calculated. The crumb and the crust were then analysed separately. The breads were also analysed once more after four days of storage.

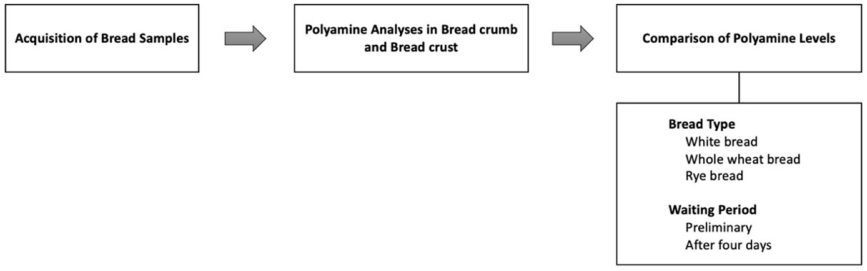

Figure 1. Schematic presentation of analysis stages

Acquisition of bread samples

The breads were collected from 10 bakeries located within the Uskudar district of Istanbul. The samples consisted of 10 loaves of each of the following bread types: white, whole wheat, and rye. The bread samples were purchased in the morning and promptly transported to the laboratory within a few hours to minimise temperature and humidity fluctuations. The experimental phase of the study was conducted in the laboratory of the Regenerative and Restorative Medicine Research Centre at Istanbul Medipol University, Türkiye, in Decem-

ber 2017. The laboratory conditions were maintained at a relative humidity of 50-60% and a temperature range of 20-24°C. The bread samples were analysed on the day of acquisition and four days after storage in the laboratory at 20-24°C and 50-60% humidity. Due to the variations observed in the products formed on the crumb and crust during the baking process, crumb and crust portions of bread samples were analysed separately.

Preparation of samples for analysis

The midpoint of the bread loaves was measured, and slices with a thickness of 2 cm were subsequently cut from this point. A second slice of the same thickness was also cut and stored in the laboratory for analysis conducted four days later. Subsequently, crumb and crust portions were separated. Samples weighing 5.0 g each from the crumb and crust portions were prepared and weighed in 50 mL Falcon tubes. The weighing of samples was performed using a precision scale (Shimadzu ATX224). To each tube, 25 mL of 1.5 M $HClO_4$ was added, and the mixture was vortexed at approximately 5-minute intervals (BioSan, V-1 Plus).

Subsequently, sonication was applied for 5 minutes in a cold ultrasonic bath (Bandelin-Sonorex, RK510). The homogenized bread sample solution underwent centrifugation at 5000 g for 10 minutes at 4 (Biocen, 22R). After centrifugation, 100 of the supernatant was transferred to a new 1.5 mL Eppendorf tube. To this, 100 of cold 1.5 M $HClO_4$ was added and mixed at moderate speed at 25°C for 30 seconds (Benchmark, H4000-HSE). Then, 100 µL of cold 2 M K_2CO_3 was added, and due to the rapid gas formation observed during this process, the procedure was completed as quickly as possible, followed by closing the cap and mixing for 10 seconds. Subsequently, the tube cap was opened, and evaporation was carried out under vacuum (Eppendorf, AG 22331). After this step, the tube caps were closed and mixed at room temperature for 30 seconds. To release any excess gas, the tube cap was opened and closed after a few seconds. The tube was then centrifuged at 15000 g for 10 minutes at 4°C (Biocen, 22R). Following this stage, the sample preparation step for High Performance Liquid Chromatography (HPLC) was initiated. The initial analysis included ten samples, while the subsequent analysis encompassed five samples.

Preparation of solutions

Mobile Phase Solution A: (0.1 M sodium acetate, pH 7.2): 27.3 g of sodium acetate (trihydrate) (Merck) and 96 µL of 6 N HCl (Merck) were dissolved in distilled water. To this solution, 180 mL methanol (Sigma Aldrich) and 10 mL of tetrahydrofuran (Sigma Aldrich) were added. The final volume was adjusted to 2 L.

Mobile Phase Solution B: A solution consisting of 100% HPLC-grade acetonitrile (Merck).

6 N HCl Solution: 49.1 mL of concentrated HCl (37%) was slowly added to 50.9 mL of distilled water and stirred.

1.5 M $HClO_4$ (perchloric acid) Solution: 32.2 mL of 70% $HCLO_4$ (Sigma Aldrich) was diluted to 250 mL with distilled water.

2 M K_2CO_3 Solution: 69.11 g of K_2CO_3 (Merck) was dissolved in 250 mL of distilled water.

1.2% (w/v) Benzoic Acid Solution: 8.4 g of benzoic acid (Sigma Aldrich) was dissolved in 525 mL of distilled water. Then, 175 mL of saturated $K_2B_4O_7$ (potassium tetraborate tetrahydrate) (Sigma Aldrich) solution was added.

40 mM Sodium Borate Buffer Solution (pH 9.5): 30.51 g of $Na_2B_4O_7.10H_2O$ (sodium tetraborate decahydrate – borax) (Merck) was dissolved in distilled water and made up to 2 litres.

Polyamine standard solutions

The solutions were prepared in water of high purity using HPLC. Polyamine standards prepared with ultrapure water in plastic tubes were stored at -80°C for no longer than 6 months.

20 mM Putrescine Solution: A 20 mM putrescine solution was prepared by dissolving 16.12 mg of putrescine.2HCl (Sigma Aldrich) (molecular weight 161.1 g/mol) in 5 mL of water. From the prepared solution, 50 µL was taken and mixed with 950 µL of ultrapure water to obtain a 1 mM putrescine standard solution.

20 mM Spermidine Solution: 25.5 mg of spermidine.3HCl (Sigma Aldrich) (molecular weight: 254.6 g/mol) were dissolved in 5 mL of water. Subsequently, 50 µL were aliquoted from the prepared solution and mixed with 950 µL of distilled water to obtain a 1 mM spermidine standard solution.

20 mM Spermine Solution: 34.9 mg of spermine.4HCl (Sigma Aldrich) (molecular weight: 348.2 g/mol) were dissolved in 5 mL of water. Subsequently, 50 µL were aliquoted from the prepared solution and mixed with 950 µL of distilled water to obtain a 1 mM spermine standard solution.

Standard mixture solution of 100 nmol/mL: 100 µL of each of the three prepared standard solutions were pipetted into an HPLC vial, followed by the addition of 700 µL distilled water.

Standard mixture solution of 10 nmol/mL: 100 μL of the 100 nmol/mL standard mixture solution was taken, followed by the addition of 900 μL of distilled water.

Analysis of the samples

For the HPLC analysis, the standard and sample solutions were prepared in vials. The standard solution was prepared by adding 750 μL of water and 50 μL of 1.2% (w/v) benzoic acid to a 2 mL plastic vial. Then, 50 μL of the standard solution was added on top. For the bread sample, 750 μL of water and 50 μL of 1.2% (w/v) benzoic acid were sequentially added to 2 mL vials. Subsequently, 200 μL of the sample was added on top. The vials were mixed for a period of 10 seconds at room temperature and then introduced into the instrument for injection. The injection volume was set to 10 μL, the injection time to 30 minutes, and the flow rate to 1.0 mL/min. Two injections were made from each sample, and the mean of the two results was calculated.

The analysis of the samples was performed using a Waters Alliance e2695 HPLC instrument equipped with a Waters 2475 FLR detector. The operating wavelengths were set to 450 nm (emission) and 340 nm (extraction). The samples were applied to a Waters WAT086344 column packed with Waters Nova-Pak C18 (150 mm length, 3.9 mm inner diameter, 4.0 μm particle size). The column temperature was maintained at 25°C.

Statistical analysis

The IBM SPSS version 22 software package (Statistical Package for Social Sciences) was employed for statistical analysis. Descriptive statistics, normality checks, and examinations through graphical and analytical methods were conducted. The dependent samples t-test, one way ANOVA, and Kruskal-Wallis analyses were employed. The results were evaluated at a significance level of 5%.

RESULTS and DISCUSSION

Comparison of polyamine level in the crumb and the crust

The level of polyamine presents in crumb and crust regions of bread (except for putrescine) was found to decrease in the following order: rye, whole wheat, and white bread, respectively. The quantities of all kinds of polyamine present in whole wheat bread were found to be significantly higher than those in white bread ($p<0.01$). Similarly, the polyamine content of rye bread was also found to be greater than that of in white bread ($p<0.01$). The spermine level in rye bread was found to be higher than that in whole wheat bread ($p<0.01$) (Table 1). It is estimated that rye bread contained the highest level of total polyamine.

Table 1. Polyamine amounts in bread crumb

Polyamines (nmol/g)	Bread Type (n=10)	Mean	SS	F/Chi* Square	p
Putrescine*	White	4.64	3.53	18.263	p<0.01 (1-2, 1-3)
	Whole wheat	26.16	24.41		
	Rye	16.16	4.31		
Spermidine*	White	16.16	12.54	18.418	p<0.01 (1-2,1-3)
	Whole wheat	46.29	17.02		
	Rye	57.43	14.17		
Spermine*	White	5.30	5.32	19.564	p<0.01 (1-2, 1-3, 2-3)
	Whole wheat	20.42	22.61		
	Rye	29.19	8.56		
Total**	White	26.11	20.64	20.201	p<0.01[a] (1-2, 1-3)
	Whole wheat	92.88	60.43		
	Rye	102.78	25.11		

1. White bread, 2. Whole wheat bread, 3. Rye bread. * Anova, ** Kruskal Wallis [a] $p<0.05$

Regarding the crust, it was observed that the levels of each kind of polyamines in rye bread were higher than those in the white bread ($p<0.01$). Moreover, the putrescine level in white bread was lower than in whole wheat ($p<0.01$). The spermine level of the rye bread was found to be significantly higher than that in the whole wheat bread ($p<0.05$) (Table 2).

Table 2. Polyamine amounts in bread crust

Polyamines (nmol/g)	Bread Type (n=10)	Mean	SS	F/Chi* Square	p[a]
Putrescine*	White	3.78	1.15	7.115	0.003 (1-2, 1-3)
	Whole wheat	7.52	3.93		
	Rye	7.38	1.49		
Spermidine*	White	9.07	4.17	11.42968	0.003 (1-3)
	Whole wheat	13.89	6.70		
	Rye	20.55	7.66		
Spermine*	White	4.79	2.37	12.01548	0.002 (1-3, 2-3)
	Whole wheat	6.56	4.38		
	Rye	12.77	5.61		
Total**	White	17.64	6.87	9.825	0.002 (1-3)
	Whole wheat	27.97	12.77		
	Rye	40.71	14.05		

1.White bread, 2. Whole bread, 3. Rye bread, * Anova, ** Kruskal Wallis, [a] $p<0.05$

The polyamine level after a four-day waiting period

This was the first study investigated the impact of staling on the polyamine content of bread. Although each polyamine varieties were predominantly found in whole bread, the differences among breads were determined to be statistically insignificant. However, the total polyamine content in whole wheat bread was found to be significantly greater than that of white bread ($p<0.05$) (Table 3).

Table 3. Polyamine amount in bread crumb after a four-day waiting period

Polyamines (nmol/g)	Bread Type (n=5)	Mean	SS	F/Chi* Square	p
Putrescine*	White	11.81	2.67	2.283	0.144
	Whole wheat	24.56	16.22		
	Rye	22.47	6.05		
Spermidine*	White	46.90	9.91	3.225	0.076
	Whole wheat	68.72	15.62		
	Rye	56.43	14.63		
Spermine*	White	24.91	4.33	3.740	0.055
	Whole wheat	35.69	9.24		
	Rye	34.75	6.22		
Total**	White	83.63	14.31	3.802	0.042[a] (1-2)
	Whole wheat	128.97	36.09		
	Rye	113.65	24.30		

1.White bread, 2. Whole wheat bread, 3. Rye bread, *Anova **Kruskal Wallis, [a]p<0.05

The results demonstrated that the variations in the levels of putrescine, spermidine, spermine, and total polyamines in the crust of bread, following a four-day waiting period, were statistically insignificant (Table 4).

Table 4. Polyamine amount in bread crust after a four-day waiting period

Polyamines (nmol/g)	Bread Type (n=5)	Mean	SS	F/Chi* Square	p
Putrescine*	White	5.37	0.31	0.527	0.603
	Whole wheat	6.51	2.59		
	Rye	6.55	2.47		
Spermidine*	White	11.64	1.90	0.757	0.490
	Whole wheat	13.78	5.91		
	Rye	9.94	5.90		
Spermine*	White	8.59	2.46	0.763	0.488
	Whole wheat	12.01	5.21		
	Rye	10.11	4.95		
Total**	White	25.59	4.13	0.600	0.564
	Whole wheat	32.31	11.76		
	Rye	26.61	13.11		

The polyamine amount of a single serving of bread

As a result, the polyamine content of 1 g of bread crumb and crust was quantified. Subsequently, the entire bread sample was weighed separately for its crumb and crust portions, enabling the calculation of the crumb-to-crust ratio. The crumb/crust ratio (%) for white, whole wheat and rye bread was estimated 68.16/31.84, 64.66/35.34 and, 58.50/41.50, respectively.

Total polyamine content of a single serving (50 g) of the rye, whole wheat and white bread were 3850.84, 3496.72 and, 1170.32 nmol of polyamines (Figure 2). Therefore, a single portion of rye bread was found to contain the highest level of polyamines. White bread was found to contain nearly three times lower than rye bread. Furthermore, it was determined that spermidine was the most abundant polyamine species in the bread.

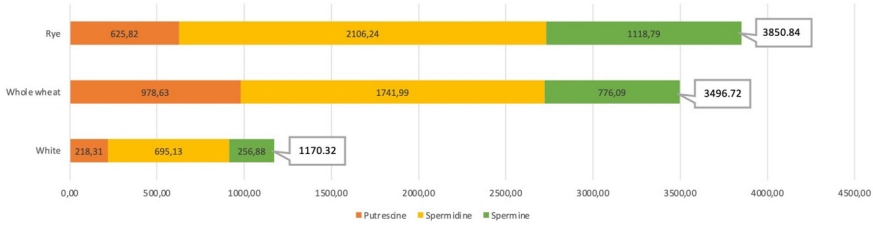

Figure 2. Polyamine content of a single portion (50 g) of bread (nmol)

Since weight and polyamine content of crumb was higher than crust, the polyamine content is greater in the crumb. It is evident that the polyamine level of the rye and whole wheat bread crumb were markedly higher in comparison to white bread. Regarding the crust, the polyamine level in the rye bread is the highest (Figure 3).

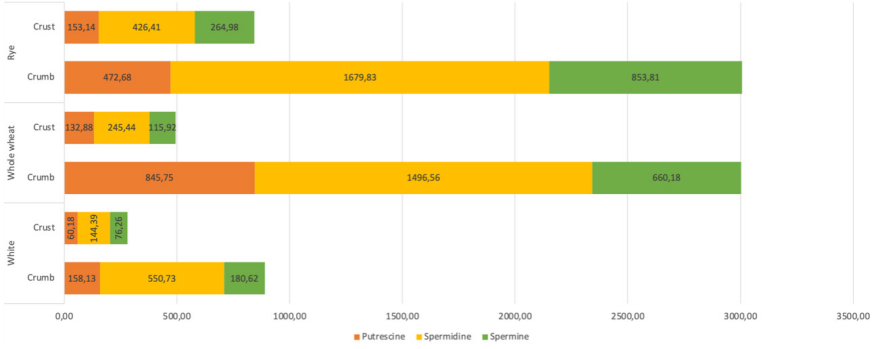

Figure 3. Polyamine content of diverse parts of bread (nmol/1 portion)

Our study was the first to perform separate polyamine analyses in bread crumb and crust, and to investigate how polyamine levels alter after storage. The study revealed that the highest level of polyamine found in both crumb and crust was spermidine, and the lowest level of polyamine was putrescine (except for whole wheat).

Baking is a complex process that involves heat and mass transfer, physical, chemical and biochemical in a product such as volume expansion, evaporation of water, formation of a porous structure, denaturation of protein, gelatinization of starch, crust formation and browning reaction. A dramatic change of physical and chemical property of dough takes place during baking process[14]. There are several ways in which crumb and crust differ from each other. The organoleptic characteristics that result from the baking process are primarily influenced by the geographical origin of the flour and the temperature at which

it is baked. The characteristics of high temperatures of the baking process result in accelerated water evaporation across the surface, leading to a reduction in water content (<20% wet basis) relative to the core[15]. In the crust, the volatile fraction is formed by thermal reactions occurring during baking process. The chemical modifications such as starch gelatinization, gluten coagulation, Maillard reactions and caramelisation of sugars affect the crust setting[16] and ingredients. We hypothesised that the polyamine content of crumb and crust differs from each other. Our results demonstrated that total polyamine levels were higher in crumb compared to crust. It could be because of insulation of the interior part by surrounding dough layers from high temperatures during the baking process. The outer parts of the dough are affected from heat more than interior parts. This causes the proofing process in the centre to continue for a while longer. The centre temperature increases independently of the oven temperature and approaches the boiling point[17]. However, the impact of the baking on polyamine content of bread has not been studied before. To date, only three studies have investigated the various cooking methods except for baking. They all found a reduction of the polyamine level. Muñoz-Esparza et al. revealed that boiling and grilling caused a significant reduction, in contrast to the effects of microwave and sous-vide methods. In the boiling, the reduction may arise from polyamine transfer to the boiling water, while high temperatures during grilling (180°C) may favour the Maillard reaction by the interaction of the primary amino groups of polyamines with reducing sugars[12]. According to Dadáková et al., stewing rabbit saddle caused 20-25% spermidine and spermine losses, while roasting and pan-roasting without oil led to 50% decrease[18]. Similarly, boiling and stewing mutton legs caused 40% losses, while roasting led to 60% reduction[19]. Consequently, high temperatures could be the driving force behind the loss of polyamines in the bread crust.

We hypothesised that the polyamine content varies between the three types of bread, since their content is different. We found that the polyamine content of whole wheat bread was greater than that in the white bread. However, the studies on the polyamine content of the bread species are strictly limited. These studies[20,21] analysed the polyamine content of the bread, however they did not include different kinds of bread. The results of Muñoz-Esparza et al.[12] and Cipolla et al.[22] are similar to our results. We also found that the level of spermidine was the highest. Similar results were reported by other studies[20,22,23]. Muñoz-Esparza et al. indicated the highest level of spermidine in wheat germ (440.6 mg/kg) among the polyamine content in cereal and derivatives. Polyamines can act as growth factors, having an important role for germination[24]. Indeed, white bread is made from the refined flour, which

constitutes 80% of the endosperm, while whole wheat flour contains the bran and the germ, which represents about 3% of the grain[12]. The higher amount of bran and the germ may lead the higher spermidine level in whole wheat bread compared to white bread. Nordlund et al. demonstrated that bread prepared with refined, whole wheat and rye flour exhibited different composition and texture characteristics. Whole wheat and rye breads contain higher level of dietary fibre compared to white bread[25]. According to Constantinescu, replacing 15% of wheat flour with rye increased the dietary fibre content 10% in rye bread[26]. Although we did not analyse the fibre in the bread, it appears that the amount of polyamine rises in accordance with the fibre content of the bread. The separation of the germ and bran from the endosperm in white bread may result in a reduction in polyamine content compared to that observed in whole wheat bread. Two studies have resulted as we suggested. Nishimura et al. reported that higher polyamine levels present in the rice bran than in the rice[21]. Karayigit et al. demonstrated that the polyamine content of whole wheat flour is higher than that of white flour[27]. However, the association between the fibre and the polyamine remains to be elucidated.

In addition to the natural presence of polyamines in foods, putrescine can also be produced through microbial[12]. Del Rio et al. found that Lactobacillus rossiae strain isolated from sourdough produces putrescine from arginine[28]. Sourdough bread is a fermented product; however, we analysed bakery-made bread produced from straight dough. The relationship between putrescine levels in bread and the yeast products used remains to be elucidated. Kobayashi et al. reported that the selection of the starter cultures with high spermidine productivity improved polyamine levels in natto, a traditional Japanese fermented soy food[29].

Our present study is the one of the first to explore the alteration of polyamine levels in bread after storage. We hypothesised that the polyamine content would alter after storage, as the quality of bread is disrupted after the production. The changes that occur aside from those influenced by microorganisms are referred to as staleness. During this process, the hardness of the bread increases, and its crumbliness is enhanced, while its water retention capacity is diminished, along with the amount of soluble starch within the bread. Crust and crumb of bread exhibit distinct behaviours. It is hypothesised that a substantial part of alterations occurs within the bread when it is taken out of oven are result of the diffusion of moisture from the surrounding air and the water present within the bread into the crust[30]. Our study revealed that the total polyamine content in both crumb and crust increased after stored four days at

20-24°C (except for rye breadcrust). However, only two studies have examined storage impact. The first showed a decline in spermine and spermidine levels in red meat and offal after eight months at -18°C and 15-20% decrease after 9 days at +2°C[18]. The second found about 20% loss of spermidine and 50% of spermine mutton loins stored at -18°C for 6 months with significant losses aerobic, vacuum-packaged or in a modified atmosphere condition at +2°C[19]. However, the storage conditions and foodstuff in our study are quite different, making comparisons difficult.

Dietary polyamines have been linked to various health benefits, including improved health and reduced mortality[31], benefits in dementia and Alzheimer's disease[32], cardiovascular health[33,34]. They may also enhance gut barrier function in short bowel syndrome[35], reduce inflammation[36], age-associated DNA methylation alterations, and tumorigenesis[37], and attenuates ischemia/reperfusion injury[38,39]. However, their role in cancer is controversial. Some studies suggest benefits for colon health[40] and oral cancer[41], while others highlight their involvement in cancer development[42-44]. Spermidine and putrescine are linked to a lower risk of type 2 diabetes, while spermine is linked to a higher risk[45]. Spermidine promotes wound healing[46], improves mitochondrial respiration and cognitive function[47], supports pulmonary system[48], offers cardioprotective effects by increasing cardiac autophagy and mitophagy, decreases blood pressure[49], and reduces lipid accumulation and core formation in atherosclerotic plaques[50]. Spermine promotes bone formation[51], and putrescine benefits the female reproductive system[52,53]. Therefore, dietary intake of polyamine is crucial for maintaining overall health. The diet provides more polyamines than the de novo biosynthesis pathway[3]. According to the recommendations of EFSA, 45-65% of energy intake should come from carbohydrates[54], richest source of which is bread and cereals. Bread is considered a staple food, contributing no less than 10% of energy requirements in the global population[10]. EU citizens are estimated to consume on average 160 g of bread per person per day, corresponding to 4-5 slices of bread[55]. In Türkiye, 39.5% of daily energy intake comes from bread and grains[11], and daily bread consumption is 134 g for women and 227 g for men[11]. Therefore, cereals and bread could contribute significantly to daily polyamine intake. In Türkiye, the daily polyamine intake is 135.899 nmol/day/person, with 12.75% coming from wheat products and 1.6–5.3% from bread[2].

This study is subject to several limitations that must be acknowledged. The utilisation of industrially produced breads rendered the ingredients unknown, and consequently, the bread production methods, such as the temperature and

duration of baking, were not considered. The genetic and cultivar characteristics of each grain were not incorporated into the study, even though they can exert a substantial influence on the dietary fibre content, as well as endosperm and germ composition of grains. The sample size may not be sufficient to capture all variations, and geographical limitations should be considered, as only bread from Istanbul was included, potentially differing from other regions. The consistency of the temperature and humidity during the storage and the separate analysis of the crumb and crust further reinforced the results. Future studies could benefit from analysing breads from different geographical region and including a wider variety of bread types. Furthermore, increasing the sample size would enhance the reliability of the findings.

In terms of human health, an increased intake of the dietary polyamine is crucial in certain health conditions. Bread plays a significant role in the diet, making it an important source of polyamines. The total polyamine content was found to be higher in the crumb than in the crust. Spermidine levels were found to be the highest across all three types of bread. The total polyamine content was estimated to increase in the following order: white, whole wheat, and rye bread, in both the crumb and crust. Regarding storage, the polyamine levels in both the crumb and crust increased after four days at 20-24°C except for rye breadcrust.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ayse Lamia Ozturk: Design study, practical performance, data analysis, preparation manuscript; Nihal Buyukuslu: Design study, data analysis, preparation manuscript, critical review manuscript; Cuneyd Parlayan: Practical performance, data analysis.

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REFERENCES

1. Handa AK, Fatima T, Mattoo AK. Polyamines: bio-molecules with diverse functions in plant and human health and disease. Front Chem, 2018;6:1-18. Doi: 10.3389/fchem.2018.00010

2. Buyukuslu N, Hizli H, Esin K, Garipagaoglu M. A cross-sectional study: nutritional polyamines in frequently consumed foods of the Turkish population. Foods, 2014;3(4):541-557. Doi: 10.3390/foods3040541

3. Kalač P. Health effects and occurrence of dietary polyamines: a review for the period 2005–mid 2013. Food Chem, 2014;161:27-39. Doi: 10.1016/j.foodchem.2014.03.102

4. Ramani D, De Bandt JP, Cynober L. Aliphatic polyamines in physiology and diseases. Clin Nutr, 2014;33(1):14-22. Doi: 10.1016/j.clnu.2013.09.019

5. Cipolla B, Guillé F, Moulinoux JP. Polyamine-reduced diet in metastatic hormone-refractory prostate cancer (HRPC) patients. Biochem Soc Trans, 2003;31(2):384-387. Doi: 10.1042/bst0310384

6. Cipolla B, Bansard JY, Ecalard JP, Moulinoux JP. Treating metastatic castration-resistant prostate cancer with novel polyamine-free oral nutritional supplementation: phase I study. BioMedicine, 2013;3(3):114-119. Doi: 10.1016/j.biomed.2013.04.005

7. Büyükuslu N. Dietary polyamines and diseases: reducing polyamine intake can be beneficial in cancer treatment. J Nutrients, 2015;2(2):27-38. Doi: 10.18488/journal.87/ 2015.2.2/87.2.27.38

8. Soda K. Overview of polyamines as nutrients for human healthy long life and effect of increased polyamine intake on DNA methylation. Cells, 2022;11(1):164. Doi: 10.3390/cells11010164

9. Jones JM, García CG, Braun HJ. Perspective: whole and refined grains and health—evidence supporting "make half your grains whole." Adv Nutr, 2019;11(3):492-506. Doi: 10.1093/advances/nmz114

10. Ribet L, Kassis A, Jacquier E, Monnet C, Durand-Dubief M, Bosco N. The nutritional contribution and relationship with health of bread consumption: a narrative review. Crit Rev Food Sci Nutr, 2024;1-28. Doi: 10.1080/10408398.2024.2428593

11. Ministry of Health of Turkey, General Directorate of Public Health. Turkey Nutrition and Health Survey (TNHS). Publication No: 1132. Ankara: Ministry of Health; 2019.

12. Muñoz-Esparza NC, Costa-Catala J, Comas-Basté O, Toro-Funes N, Latorre-Moratalla ML, Veciana-Nogués MT, et al. Occurrence of polyamines in foods and the influence of cooking processes. Foods, 2021;10(8):1752. Doi: 10.3390/foods10081752

13. Binh PNT, Soda K, Maruyama C, Kawakami M. Relationship between food polyamines and gross domestic product in association with longevity in Asian countries. Health, 2010;2(12):1390-1396. Doi: 10.4236/health.2010.212206

14. Dessev T, Lalanne V, Keramat J, Jury V, Prost C, Le-Bail A. Influence of baking conditions on bread characteristics and acrylamide concentration. J Food Sci Nutr Res, 2020;3(4):291-310. Doi: 10.26502/jfsnr.2642-11000056

15. Vanin FM, Lucas T, Trystram G. Crust formation and its role during bread baking. Trends Food Sci Technol, 2009;20(8):333-343. Doi: 10.1016/j.tifs.2009.04.001

16. Cho IH, Peterson DG. Chemistry of bread aroma: a review. Food Sci Biotechnol, 2010;19(3):575-582. Doi: 10.1007/s10068-010-0081-3

17. Cauvain SP. Breadmaking: an overview. In: Cauvain SP, editor. Breadmaking: improving quality. 2nd ed. Cambridge: Woodhead Publishing; 2012. p. 8-28. Doi: 10.1533/9781855737129.8

18. Dadáková E, Pelikánová T, Kalač P. Concentration of biologically active polyamines in rabbit meat, liver and kidney after slaughter and their changes during meat storage and cooking. Meat Sci, 2012;90(3):796-800. Doi: 10.1016/j.meatsci.2011.11.017

19. Dadáková E, Pelikánová T, Kalač P. Concentration of biologically active polyamines in meat and liver of sheep and lambs after slaughter and their changes in mutton during storage and cooking. Meat Sci, 2011;87(2):119-124. Doi: 10.1016/j.meatsci.2010.09.009

20. Nishibori N, Fujihara S, Akatuki T. Amounts of polyamines in foods in Japan and intake by Japanese. Food Chem, 2007;100(2):491-497. Doi: 10.1016/j.foodchem.2005.09.070

21. Nishimura K, Shiina R, Kashiwagi K, Igarashi K. Decrease in polyamines with aging and their ingestion from food and drink. J Biochem, 2006;139(1):81-90. Doi: 10.1093/jb/mvj003

22. Cipolla BG, Havouis R, Moulinoux JP. Polyamine contents in current foods: a basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients. Amino Acids, 2007;33(2):203-212. Doi: 10.1007/s00726-007-0524-1

23. Eliassen KA, Reistad R, Risoen U, Ronning HF. Dietary polyamines. Food Chem, 2002;78(3):273-280. Doi: 10.1016/S0308-8146(01)00405-8

24. Chen D, Shao Q, Yin L, Younis A, Zheng B. Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Front Plant Sci, 2019;9:429326. Doi: 10.3389/fpls.2018.01945

25. Nordlund E, Katina K, Mykkänen H, Poutanen K. Distinct characteristics of rye and wheat breads impact on their *in vitro* gastric disintegration and *in vivo* glucose and insulin responses. Foods, 2016;5(2):24. Doi: 10.3390/foods5020024

26. Pop G. Quality of high-fiber wheat bread improved with rye flour. J Agroaliment Process Technol, 2009;15(2):287-292.

27. Karayigit B, Colak N, Ozogul F, Gundogdu A, Inceer H, Bilgiçli N, et al. The biogenic amine and mineral contents of different milling fractions of bread and durum wheat (*Triticum* L.) cultivars. Food Biosci, 2020;37:100676. Doi: 10.1016/j.fbio.2020.100676

28. Del Rio B, Alvarez-Sieiro P, Redruello B, Martin MC, Fernandez M, Ladero V, et al. *Lactobacillus rossiae* strain isolated from sourdough produces putrescine from arginine. Sci Rep, 2018;8:3989. Doi: 10.1038/s41598-018-22309-6

29. Kobayashi K, Shimojo S, Watanabe S. Contribution of a fermentation process using *Bacillus subtilis* (natto) to high polyamine contents of natto, a traditional Japanese fermented soy food. Food Sci Technol Res, 2016;22(1):153-157. Doi: 10.3136/fstr.22.153

30. Gerçekaslan KE, Kotancılar HG, Karaoğlu MM, Ertugay MF. Ekmek bayatlaması ve bayatlama derecesini ölçmede kullanılan yöntemler: II. Gıda, 2008;33(1):27-34.

31. Madeo F, Hofer SJ, Pendl T, Bauer MA, Eisenberg T, Carmona-Gutierrez D, et al. Nutritional aspects of spermidine. Annu Rev Nutr, 2020;40:135-159. Doi: 10.1146/annurev-nutr-120419-015419

32. Qian M, Zhang N, Zhang R, Liu M, Wu Y, Lu Y, et al. Non-linear association of dietary polyamines with the risk of incident dementia: results from population-based cohort of the UK Biobank. Nutrients, 2024;16(16):2774. Doi: 10.3390/nu16162774

33. Han S, Qian M, Zhang N, Zhang R, Liu M, Wang J, et al. The association of dietary polyamines with mortality and the risk of cardiovascular disease: a prospective study in UK Biobank. Nutrients, 2024;16(24):4335. Doi: 10.3390/nu16244335

34. Soda K, Kano Y, Chiba F. Food polyamine and cardiovascular disease – an epidemiological study. Glob J Health Sci, 2012;4(6):170-178. Doi: 10.5539/gjhs.v4n6p170

35. Kasahara N, Teratani T, Yokota S, Sakuma Y, Sasanuma H, Fujimoto Y, et al. Dietary polyamines promote intestinal adaptation in an experimental model of short bowel syndrome. Sci Rep, 2024;14(1):4605. Doi: 10.1038/s41598-024-55258-4

36. Soda K, Uemura T, Sanayama H, Igarashi K, Fukui T. Polyamine-rich diet elevates blood spermine levels and inhibits pro-inflammatory status: an interventional study. Med Sci (Basel), 2021;9(2):22. Doi: 10.3390/medsci9020022

37. Soda K, Kano Y, Chiba F, Koizumi K, Miyaki Y. Increased polyamine intake inhibits age-associated alteration in global DNA methylation and 1,2-dimethylhydrazine-induced tumorigenesis. PLoS One, 2013;8(5):e64357. Doi: 10.1371/journal.pone.0064357

38. Doi J, Fujimoto Y, Teratani T, Kasahara N, Maeda M, Tsuruyama T, et al. Bolus administration of polyamines boosts effects on hepatic ischemia-reperfusion injury and regeneration in rats. Eur Surg Res, 2019;60(1-2):63-73. Doi: 10.1159/000497434

39. Okumura S, Teratani T, Fujimoto Y, Zhao X, Tsuruyama T, Masano Y, et al. Oral administration of polyamines ameliorates liver ischemia/reperfusion injury and promotes liver regeneration in rats. Liver Transplant, 2016;22(9):1231-1244. Doi: 10.1002/lt.24471

40. Vargas AJ, Ashbeck EL, Wertheim BC, Wallace RB, Neuhouser ML, Thomson CA, et al. Dietary polyamine intake and colorectal cancer risk in postmenopausal women. Am J Clin Nutr, 2015;102(2):411-419. Doi: 10.3945/ajcn.114.103895

41. Coeli-Lacchini FB, da Silva G, Belentani M, Alves JSF, Ushida TR, Lunardelli GT, et al. Spermidine suppresses oral carcinogenesis through autophagy induction, DNA damage repair, and oxidative stress reduction. Am J Pathol, 2023;193(12):2172-2181. Doi: 10.1016/j.ajpath.2023.09.005

42. Vargas AJ, Wertheim BC, Gerner EW, Thomson CA, Rock CL, Thompson PA. Dietary polyamine intake and risk of colorectal adenomatous polyps. Am J Clin Nutr, 2012;96(1):133-141. Doi: 10.3945/ajcn.111.030353

43. Huang CY, Fang YJ, Abulimiti A, Yang X, Li L, Liu KY, et al. Dietary polyamines intake and risk of colorectal cancer: a case-control study. Nutrients, 2020;12(11):3575. Doi: 10.3390/nu12113575

44. Arisan ED, Çoker A, Palavan-Ünsal N. Polyamine depletion enhances the roscovitine-induced apoptosis through the activation of mitochondria in HCT116 colon carcinoma cells. Amino Acids, 2012;42(2-3):655-665. Doi: 10.1007/s00726-011-1040-x

45. Zhang X, Qian M, Liu M, He M, Li FR, Zheng L. The associations of dietary polyamines with incident type 2 diabetes mellitus: a large prospective cohort study. Nutrients, 2025;17(1):186. Doi: 10.3390/nu17010186

46. Ito D, Ito H, Ideta T, Kanbe A, Ninomiya S, Shimizu M. Systemic and topical administration of spermidine accelerates skin wound healing. Cell Commun Signal, 2021;19(1):36. Doi: 10.1186/s12964-021-00717-y

47. Schroeder S, Hofer SJ, Zimmermann A, Pechlaner R, Dammbrueck C, Pendl T, et al. Dietary spermidine improves cognitive function. Cell Rep, 2021;35(2):108985. Doi: 10.1016/j.celrep.2021.108985

48. Chen YJ, Li HF, Zhao FR, Yu M, Pan SY, Sun WZ, et al. Spermidine attenuates monocrotaline-induced pulmonary arterial hypertension in rats by inhibiting purine metabolism and polyamine synthesis-associated vascular remodeling. Int Immunopharmacol, 2024;132:111946. Doi: 10.1016/j.intimp.2024.111946

49. Eisenberg T, Abdellatif M, Zimmermann A, Schroeder S, Pendl T, Harger A, et al. Dietary spermidine for lowering high blood pressure. Autophagy, 2017;13(4):767-769. Doi: 10.1080/15548627.2017.1280225

50. Michiels CF, Kurdi A, Timmermans JP, De Meyer GRY, Martinet W. Spermidine reduces lipid accumulation and necrotic core formation in atherosclerotic plaques via induction of autophagy. Atherosclerosis, 2016;251:319-327. Doi: 10.1016/j.atherosclerosis.2016.07.899

51. Jiang RX, Hu N, Deng YW, Hu LW, Gu H, Luo N, et al. Potential therapeutic role of spermine via Rac1 in osteoporosis: Insights from zebrafish and mice. Zool Res, 2024;45(2):367-380. Doi: 10.24272/j.issn.2095-8137.2023.371

52. Tao Y, Tartia A, Lawson M, Zelinski MB, Wu W, Liu JY, et al. Can peri-ovulatory putrescine supplementation improve egg quality in older infertile women? J Assist Reprod Genet, 2019;36(3):395-402. Doi: 10.1007/s10815-018-1327-x

53. Liu D, Mo G, Tao Y, Wang H, Liu XJ. Putrescine supplementation during *in vitro* maturation of aged mouse oocytes improves the quality of blastocysts. Reprod Fertil Dev, 2017;29(7):1392-1400. Doi: 10.1071/RD16061

54. European Food Safety Authority. EFSA sets European dietary reference values for nutrient intakes. *EFSA* [Internet]. European Food Safety Authority; 2020 [Feb 24, 2025]. Available from: https://www.efsa.europa.eu/en/press/news/nda100326

55. Association Internationale de la Boulangerie Industrielle (AIBI). AIBI bread market report 2013. AIBI; 2015.