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The effect of the stale bread flour addition on flour and bread quality

https://doi.org/10.1515/ijfe-2019-0100
Received March 17, 2019; accepted August 22, 2020; published online September 7, 2020

Abstract: In this study, the effect of the flour, which was obtained from stale bread, on flour and bread quality was investigated. The bread that was staled at refrigerator for 7 days was prepared as whole and crumb, and was grinded to convert into flour. The staled whole and crumb bread flours were stored at −18 °C and used to replace 0, 15, 30 and 45% of wheat flour. Then microbiological and physicochemical properties of flours; physical, textural and sensory properties of bread obtained from these composite flours were investigated. We concluded that stale bread flour could be used for bread production at the level of 15%. If the total bread production and consumption is considered, this addition level could provide a significant amount of waste bread recycling each year.

Keywords: bread; consumption quality; stale bread; textural properties.

1 Introduction

Bread has a major part in daily diet and is consumed by most people almost all over the world [1, 2]. It provides good amount of energy and nutrients because of containing carbohydrates, proteins, lipids, fibres, minerals and vitamins [3]. Bakery products mostly have a short shelf-life because of staling and microbiological spoilage. Staling leads to some undesirable changes like lessening in freshness, loss of aroma and flavour and increased hardness of the crumb [4]. The quality and consumer acceptance of bread considerably decrease because of these changes. Staling is one of the most prevalent reasons of bread waste, which causes important economic loss [5, 6].

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Microbiological spoilage and staling are dominant factors that affect the quality of bread [7]. Bread starts staling immediately after production and consequently loses significant sensory parameters like texture, flavour and freshness. Staling causes moisture redistribution from crumb to crust, and as a result of that, gelatinized starch becomes re-crystallized (retrogradation) [8, 9]. Staling is a complex mechanism and has not been completely understood until now [10, 11]. Although numerous studies have been conducted to deal with staling, satisfactory solutions have not been found yet [12].

Along with staling, microbiological spoilage, which is also one of the major factors that limit the shelf life of bread, occurs. Fungi (*Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp.) and heat resistant spore-forming bacteria (*Bacillus* spp. particularly *Bacillus subtilis* and *Bacillus licheniformis*) are the two main reasons, which cause microbiological spoilage [13–15]. Fungal growth is the most important spoiler agent that determines the shelf life of bakery products [7]. While *B. subtillis* spores occur in raw materials and survive to the baking temperature, fungal spoilage happens as post-processing contamination [14, 16]. Although world population is increasing, one-third of the edible parts of food is wasted annually [17]. According to a study conducted in Turkey, around 5 million breads staled per day, which means 228 million Dollars per year [18]. Since the stalling mechanism can only be delayed to some extent, bread that is not consumed due to staling and significant economic losses stemming from it are important issues to be solved by researchers working in this field. From this point of view, there is considerable interest in recycling stale bread to produce commercially valuable products. In this regard, various studies have been conducted in which ethanol production [19, 20], bio-hydrogen production [21, 22], feedstuff production for pigs [23], succinic acid production [24], amylase production [25], glucoamylase and protease production [26], baker yeast production [27], starter dough for baking baked wheat products [28] and wheat chips production which is enriched with stale bread [1], were tried. Although there are plenty of data available in the literature for recycling bread to various products, converting stale bread into flour and using it again in bread production has not been tried. This study therefore focused on investigating the possibility of

recycling stale bread, which was grinded like flour, by adding them to bread formulations at different levels. By investigating the textural, microbiological and physicochemical properties of the bread produced in this way, the effect of stale bread flour on these properties were studied and the possibility of using stale bread flour in bread production was examined.

2 Material and methods

2.1 Material

The commercial wheat flour (Ozlu Un, Erzurum, Turkey), fresh yeast (*Saccharomyces cerevisiae*) of Pakmaya (Besiktas, Istanbul, Turkey) and salt were bought from local supermarkets in Erzurum.

2.1.1 Stale bread flour production: Dough was prepared by 100 g flour, 1.5% salt, 2.5% fresh yeast and 56.6% water based on the flour weight. Bread was produced according to AACC 10-10.03 method [29], with some modifications. All dough ingredients were kneaded with Stephan UM-5 kneader (Hameln, Germany). Dough were divided into portions and weighed for 160 g then rolled. After the fermentation step, the divided dough portions were manually moulded and placed in a baking tray. The moulded dough portions were baked in the preheated oven for 25 min at 230 °C. After 1 h cooling step, breads were stored in polyethylene bags for 7 days at refrigerator temperature (4 °C). Half of the stale bread crusts were separated to get crumb. In order to obtain a grindable structure for the stored breads, both crumb and whole loaves of bread were cut into 5 mm^3 pieces and dried at 40 °C until the moisture content was reduced to about 10%. Dried bread pieces were grinded with Buhler Laboratory Mill (Buhler, Switzerland) and passed through the 160 μm sieve to obtain stale bread flour. The stale bread flour was packed with polyethylene bags and kept in deep-freezing (–18 °C) until use. Stale Crumb Bread Flour (SCBF) and Stale Whole Bread Flour (SWBF) were used at different levels (15, 30, 45%), based on the flour weight, in the production of bread. The control bread sample was prepared using wheat flour without the addition of stale bread flour.

2.2 Method

2.2.1 Wheat flour and stale bread flour analyses

2.2.1.1 Microbiological analyses: Diluted flour and stale bread flour were inoculated according to spreading method on Potato Dextrose Agar (PDA) (Merck) medium for the mould and yeast counts. Petri plates were incubated at 30 °C for 3–5 days [30]. Plate Count Agar (PCA) (Merck) were used for counting Total Aerobic Mesophilic Bacteria (TAMB) according to the spreading method. Petri plates were incubated at 30 °C for 3 days [31]. Flour homogenate was transferred to sterile test tubes with sterile saline solution and was kept at 80 °C for 10 min for detection of *Bacillus* spores. The tubes were cooled to 45–50 °C and dilutions were inoculated according to spreading method on Plate Count Agar (PCA) (Merck). Petri plates were incubated for 3 days at 30 °C [32]. At the end of the incubation time, the number of the Colony forming Units (CFU) was counted.

2.2.1.2 Evaluation of physicochemical properties of flour: Zeleny Sedimentation value of wheat flour was determined on the basis of 14% humidity using a mechanical shaker [33]. The activity of the α-amylase enzyme of wheat flour was determined by the Falling Number (Perten Falling Number 1500, Sweden) [34]. The pH values of the wheat and stale bread flour samples were measured according to AACC 02-52.01 method by a pH meter (Hanna Instruments, Italy) [35].

2.2.2 Breadmaking process: Bread samples were produced according to AACC 10-10.03 method [29], with some modifications. Mixed ingredients were: 1000 g of flour, 566 g of water, 25 g of yeast, and 15 g of salt and stale bread flour at different rates (0, 15, 30 and 45% flour basis). All ingredients were kneaded with Stephan UM-5 kneader (Hameln, Germany) for 2 min. The dough samples were divided into 160 g portions that were manually moulded. After fermentation steps (60 min, at 30 °C, 85% Relative Humidity) dough was panned by shaping before proofing for 40 min at 30 °C (85% Relative Humidity). The shaped dough portions were baked in the pre-heated oven for 25 min at 230 °C. The produced bread types were stored in polyethylene bags at room temperature (20 °C) until analysis time.

2.2.3 Bread characterization

2.2.3.1 Specific volume and weight loss: Following the production, bread samples were weighted, and each volume was determined by rapeseed displacement after 1 h of cooling. The specific volume was calculated as volume/bread weight (cm^3/g). Weight loss was calculated according to water loss amount from bread and expressed as percentage (%). Measurements were performed by three replicates [36].

2.2.3.2 Bread crust colour: Bread crust colour was measured using a colourimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan; light source D65) according to International Commission on Illumination formula. This formula is based on a three-dimensional colour measurement; L (lightness, 0=black 100=White), a (+ indicates redness, and – greenness) and b (+ indicates yellowness, and – blueness) [37]. Five readings were made at different positions from the top surface of the breads for colour measurement. The mean value for L, a and b were recorded. Total colour change (ΔE) was calculated according to the following equations:

$$\Delta E = \left[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2 \right]^{1/2}$$

Where L_0, a_0 and b_0 are colour parameter values of the control bread and L, a and b are colour parameter values of the stale bread flours added samples. The above equation was used in order to estimate the colour differences in stale bread flour added bread samples as compared to control bread.

2.2.3.3 Crumb texture profile analysis: Texture Profile Analysis (TPA) of bread crumb was evaluated with SMS Texture Analyser (model TA-XT.plus, Stable Micro System, England) using a 50 mm diameter probe, after 12 h of storage at 20 °C. Bread crumb samples were prepared from the middle parts of the bread in a special slicing cabin with 2.5 × 2.5 × 2.5 cm height, width and depth. The determined parameters were hardness, adhesiveness, cohesiveness, springiness and chewiness. The conditions under which the breadcrumb TPA method was pre-test speed 2.00 mm/s, test speed 1.00 mm/s, post-test speed 1.00 mm/s, compression rate %50.00 and trigger force 10.00 g.

2.2.3.4 Acrylamide content: The Acrylamide content of the Control and 45% SWBF added bread samples were analysed according to Rufian-Henares & Morales [38] Method with Agilent ll00 liquid chromatograph coupled to an Agilent Quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA).

2.2.3.5 Sensory characterisation: Sensory characterisation was carried on using the hedonic scale according to Anzaldua-Morales [39] method from 0 to 15 where one is 'quite disliked', seven is 'neither liked nor disliked', and 15 is 'quite liked'. Assessors were randomly selected ($n = 25$) from Atatürk University students and staff. Each panel member evaluated the breads for five sensory characteristics including crumb colour, pore structure, hardness, aroma and overall acceptability.

2.2.4 Statistical analysis: Data were obtained as mean ± standard error, followed by analysis of variance using the SPSS (SPSS for Windows Release 10.0.1, 1999). The mean of the significant values of variance were compared by Duncan's Multiple-Range Test ($P < 0.01$) using the SPSS [40].

3 Results and discussion

3.1 Wheat flour and stale bread flour analyses

3.1.1 Microbiological analyses

Mould and yeast, Total Aerobic Mesophilic Bacteria (TAMB), and *Bacillus* spore counts were expressed in Colony Forming Units (CFU) per gram of flour in Table 1. The results obtained in microbiological analysis were suitable for all stale bread flours according to the Turkish Food Codex Regulation on Microbiological Criteria [41]. The moisture content of stale whole and crumb bread flours were less than 10%, which is not appropriate for growing microorganism groups. The number of TAMB in stale whole and crumb flours were less than control flour. Yeast and mould counts of control and stale bread flours were close to the control flour. *Bacillus* spores are resistant to heat and can survive during the baking process [14]. In accordance with this information, the number of *Bacillus* spores in stale bread flours were similar with control flour.

Table 1: Microbiological analysis results of stale bread flour.

Staling duration	Bread part	Mould &yeast (cfu/g)	*Bacillus* spores (cfu/g)	TAMB (cfu/g)
Control flour	–	$<10^3$	10^3	$1.9x10^4$
7 (Days refrigerator temp.)	Whole	10^3	10^3	$6x10^3$
	Crumb	10^4	2.5×10^3	$1.3x10^3$

3.1.2 Evaluation of physicochemical properties of flour

Control flour was found to have a pH of 6.29. When compared with the wheat flour, the pH value of SCBF and SWBF were decreased to 6.04 and 5.88, respectively at a significant level. Falling Number values, which is a sign of the alpha-amylase enzyme activity, were found 62 s in all stale bread flours while it was found 578 s at the wheat flour. Zeleny sedimentation values, which gives an idea of the gluten quality of flour, were found 12.5, 13.0 and 37.0 for SWBF, SCBF and wheat flour respectively. The heat treatment applied during baking denaturates the proteins found in the structure especially the gluten, and therefore, causes decreases in Zeleny sedimentation values.

3.2 Bread characterization

3.2.1 Specific volume and weight loss

Figure 1 shows specific volume and weight loss of bread samples, to which added SWBF and SCBF were added at different rates (15, 30 and 45%), compared to the Control Bread. Figure 2 illustrates the photos of these bread samples. Specific volume and weight loss of bread samples decreased with the SWBF and SCBF addition at all levels (Figure 2). When all quality parameters of bread are considered, specific volume is strongly related to the gluten network quality and CO_2 holding capacity [42]. Gluten has lost its dough-forming (visco-elastic structure) property because it was denatured by heat in stale bread flour. Therefore, the increase in the level of stale flour addition affected the properties of the dough in a negative way, and this was reflected in the properties (such as the specific volume of the bread) obtained from these doughs. Specific volume is highly related to the Zeleny Sedimentation in terms of gluten quality. Adding over 15% of the stale bread flour decreased the specific volume at a significant level. This decrease caused increase in hardness values, which is one of the TPA parameters, of stale flour added bread samples (Figure 3).

3.2.2 Bread crust colour

Crust colour is another important quality characteristic of bread when consumer expectation is considered [43]. The addition level of stale bread flour significantly affected all colour parameters of bread crust (L, a, b and ΔE) (Table 2). L colour value (lightness) of all bread samples was lower than the control except the sample to which SCBF was added at a rate of 45%. However, as addition level increased, L colour value increased. The closest L colour

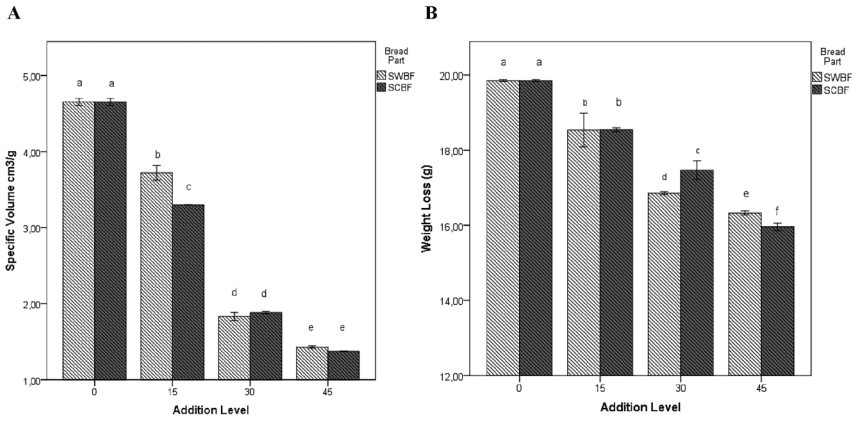


Figure 1: Effect of the interaction of addition levels *x* variety of stale bread flour (SWBF, SCBF) on specific volume (A) and weight Loss (B) properties of bread samples after cooling 1 h at 20 °C. Different letters within the same group of bars indicate significant differences ($P < 0.05$).



Figure 2: Control, SWBF (Stale Whole Bread Flour) and SCBF (Stale Crumb Bread Flour) added breads photos.

value to the control was bread sample with 45% SWBF. When the SWBF addition to the formulation was increased up to 30%, a regular increase in the *a* colour value (redness) was observed, but a sudden decrease was detected when this level increased to 45%. *a* colour value of bread samples decreased with increasing of the SCBF. *a* colour value of bread samples to which SWBF was added at a rate of 30 and 45% was higher than bread samples to which SCBF was added. However, *a* colour value of bread samples with 15% SWBF and 15% SCBF were found to be close to each other. Crust colour of bread mainly formed through nonenzymatic browning reactions such as caramelization and maillard that occur relatively slow in high moisture foods [44, 45]. Adding both SWBF and SCBF at a rate of 45% caused decreases in the specific volume and weight loss, and the high moisture in bread restricted the nonenzymatic browning reactions on bread crust (Figure 1). Therefore, both SWBF and SCBF addition at 45% significantly decreased the *a* colour value of bread crust. *b* colour value (yellowness) of bread samples decreased with the addition of 15% stale bread flour while it increased in the samples produced with 30 and 45% stale bread flour compared to the control. In general, the differences in the colour values of stale bread flours seem to be reflected in the colour values of the bread samples produced by using these stale bread flours. For instance, *L* colour value of

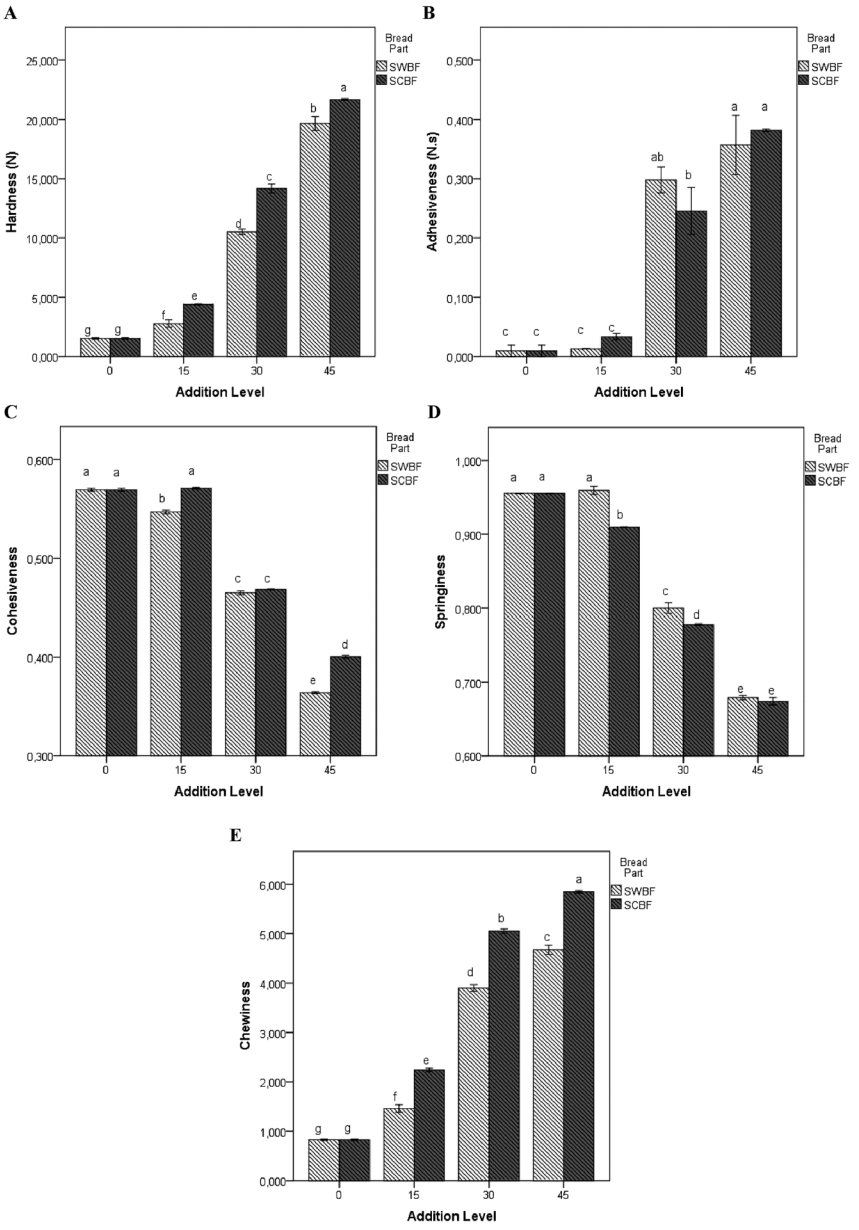


Figure 3: 0: control Crumb hardness (A), adhesiveness (B), cohesiveness (C), springiness (D), and chewiness (E) for bread. Different letters within the same group of bars indicate significant differences ($P < 0.05$).

SWBF was lower than the control, for this reason, SWBF addition at all levels caused to decrease in the L value of samples. In all SWBF and SCBF added samples there were significant differences in crust colour ΔE values compared to the control bread. Bread samples with 15%, 45% SCBF and 15% SWBF received ΔE values higher than bread samples with 30% SWBF, 30% SCBF, and %45 SWBF respectively. The closest ΔE value to control was SWBF was added at a rate of 45%.

3.2.3 Crumb texture profile analysis

Texture plays a vital role in affecting sensory perception and shelf life of bread [46]. The effects of SWBF and SCBF addition on the texture parameters are shown in Figure 3. The increase in the stale bread flour level caused increases in the hardness, adhesiveness, and chewiness parameters, and caused decreases in the springiness and cohesiveness values of bread. In most bakery products like bread, retrogradation of starch causes increased textural hardness and moisture loss, and these changes become progressively worse and more noticeable as the product ages [47]. The highest hardness value was found in samples with 45% SCBF while the lowest hardness value was found in samples to which SWBF was added at the level of 15% compared to the control. Similarly, Rosell & Santos [48], found that adding the resistant starch and fibre to wheat bread resulted in a significant increase in the crumb

Table 2: Colour analyse results of final breads.

Bread part	Stale bread flour rate (%)				
					Bread crust colour
		L	+*a*	+*b*	Δ*E*
	0 (C)	58.55 ± 0.66b	11.41 ± 0.34b	21.34 ± 1.54b	–
SWBF	15	49.98 ± 0.26c	13.95 ± 0.77b	19.52 ± 1.71c	10.99 ± 0.92b
	30	50.24 ± 0.17c	15.05 ± 0.43a	26.27 ± 0.42b	10.32 ± 0.18bc
	45	57.74 ± 0.03b	6.42 ± 0.80c	24.96 ± 1.40b	6.1 ± 0.19d
SCBF	15	47.51 ± 0.05d	13.98 ± 1.03b	18.69 ± 1.32c	13.04 ± 0.41a
	30	57.51 ± 0.84b	11.70 ± 0.54b	31.87 ± 0.25a	9.34 ± 0.20c
	45	65.83 ± 0.06a	3.23 ± 0.10d	26.44 ± 0.15b	11.44 ± 0.09b
P		**	**	**	**

C, Control; SWBF, Stale Whole Bread Flour; SCBF, Stale Crumb Bread Flour; [a,b,c,d] Different letters mean statistical difference at $P < 0.01$ level.

hardness. Scheuer et al. [49], also reported that adding dietary fibres to the wheat flour could disrupt starch-gluten matrix and limit the CO_2 holding capacity by eventually affecting specific volume and hardness. The addition of SWBF and SCBF significantly increased the adhesiveness of bread samples. Weight loss of all samples decreased with the increasing of both SWBF and SCBF addition. This shows that increase in the addition level of SWBF and SCBF limits the swelling capacity of dough (Figure 1). Specific volume of bread samples to which SWBF and SCBF were added was lower than control because of low weight loss values. Low weight loss in SWBF and SCBF added bread samples caused high moisture content, and consequently adhesiveness value of those samples were found to be higher than control. The SWBF and SCBF that was added more than 15% decreased the cohesiveness of the bread samples. Cohesiveness value of breads with SWBF decreased more than in breads with SCBF. The springiness values of both SCBF and SWBF added bread samples showed a decreasing trend when the addition level was increased. Chewiness value of all bread samples was increased with the increasing of both SWBF and SCBF. Verdú et al. [50], also reported that substituting the wheat flour with wheat bran and chia seeds resulted in increasing of the hardness, chewiness and decreasing of the cohesiveness, springiness values of breads. When all texture parameters were considered, closest result to the control was found in the bread to which 15% SWBF was added.

3.2.4 Acrylamide content

Acrylamide content of control and bread samples to which 45% SWBF were added was found to be 0.051 and 0.045 mg/kg, respectively. According to International Agency for Research on Cancer (IARC), acrylamide is classified as group 2A (probably carcinogenic to human) and it is generated as a result of heat treatment [51]. Claus et al. [52], reported that more than 99% acrylamide was found in the bread crust. Acrylamide is not formed at temperatures below 100 °C during baking so bread crumb contains trace amount of acrylamide. From this information, in this study we analysed control and bread samples to which 45% SWBF was added (max. addition level). It is stated that the acrylamide content in bread may be in a very

Table 3: Sensory evaluation of bread samples.

Bread part	Stale bread flour rate (%)	Crumb colour	Pore structure	Hardness	Aroma	Overall acceptability
	0 (C)	5 ± 0.50e	13 ± 0.50bc	2 ± 0.50d	13 ± 0.50a	14 ± 0.50a
SWBF	15	7 ± 0.50cd	14 ± 0.50a	3 ± 0.50d	13 ± 0.50a	14 ± 0.50a
	30	9 ± 0.50ab	12 ± 0.50bc	8 ± 0.50c	12 ± 0.50a	10 ± 0.50c
	45	10 ± 0.50a	8 ± 0.50e	11 ± 0.50b	8 ± 0.50c	2 ± 0.50d
SCBF	15	7 ± 0.50cd	13 ± 0.50ab	7 ± 0.50c	13 ± 0.50a	13 ± 0.50a
	30	8 ± 0.50bc	11 ± 0.50c	8 ± 0.50c	13 ± 0.50a	11 ± 0.50b
	45	9 ± 0.50ab	9 ± 0.50d	13 ± 0.50a	9 ± 0.50b	2 ± 0.50d
P		**	**	**	**	**

C, Control; SWBF, Stale Whole Bread Flour; SCBF, Stale Crumb Bread Flour; [a,b,c,d,e] Different letters mean statistical difference at $P < 0.01$ level.

wide range of 0.02 – 0.250 mg/kg [53, 54]. Acrylamide content of control and bread samples to which 45% SWBF was added was found to be lower than the intervals stated in the literature.

3.2.5 Sensory characterisation

In general, the addition of stale bread flour at higher addition level (30%, 45%) resulted in decreased scores for pore structure, and overall acceptability values while increased scores for crumb colour, and hardness values at all addition level (15, 30, 45%) compared to the control (Table 3). When addition level of both SWBF and SCBF was at 15 and 30%, aroma scores of breads were close to the control. Whereas a sudden decrease was detected when this level increased to 45%. According to overall acceptability scores, the bread to which the SWBF was added at a rate of 15% was liked the most; and the samples to which both SWBF and SCBF were added at a rate of 45% were the ones that were least liked. Addition of stale bread flours at higher levels mostly caused less acceptable sensory parameters.

4 Conclusion

In this study, the possibility of using stale bread, which is substantially wasted every year all over the world in bread production, was researched. For this aim, flours were obtained from stale breads, and then the qualitative characteristics were examined in bread samples produced by using these flours. Addition of stale bread flour to bread decreased the *L* colour value, and generally increased the *a* and *b* colour values. The increase in the addition level of stale bread flour caused increases in the hardness, adhesiveness, and chewiness parameters, and caused decreases in the springiness and cohesiveness values. Addition of stale bread flour to bread formulation could disrupt starch-gluten matrix and limit the CO_2 holding capacity, and eventually affect specific volume and hardness. Sensory analysis results show that the bread to which the SWBF was added at a rate of 15% was liked the most; and the samples to which both SWBF and stale SCBF were added at a rate of 45% were the ones that were least liked. In summary, the results found in this study demonstrate that stale bread flour could be used for bread production at a rate of 15%. If the total bread production and consumption is considered, this addition level could provide a significant amount of waste bread recycling each year.

Acknowledgments: We would like to thank Miss. Rezvan SHIEHZADE for technical assistance. This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK), the Project number of 215O644.

Abbreviations and Nomenclature

SWBF	Stale Whole Bread Flour
SCBF	Stale Crumb Bread Flour
min	Minutes
N	Newton
TPA	Textural profile analysis
CO_2	Carbon dioxide
L	Lightness
b	Yellowness
a	Redness
TAMB	Total Aerobic Mesophilic Bacteria
CFU	Colony Forming Units
PCA	Plate Count Agar
PDA	Potato Dextrose Agar

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK), the Project number of 215O644.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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