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Research Article

Effect of Exopolysaccharide Extracted from Iranian Kefir Grains on Bread Quality Properties of Medium Protein Wheat

Mansooreh Soleimani Fard[1]*, Mehran Aalami[2] and Alireza Sadeghi Mahoonak[2]

[1]Ph.D student of food science-food chemistry, Department of Food Science and Technology, University of Agricultural Sciences and Natural Resources of Gorgan, Gorgan, Iran

[2]Faculty Member of department Food Science and Technology, University of agricultural Sciences and Natural Resources of Gorgan, Gorgan, Iran

***Corresponding Author**: Mansooreh Soleimani Fard, Ph.D student of food science-food chemistry, Department of Food Science and Technology, University of Agricultural Sciences and Natural Resources of Gorgan, Gorgan, Iran.

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Abstract

This research discussed the impact of kefiran application, an exopolysaccharide extracted from kefir grains, as a new improver material. Kefiran was added at 1, 2, 3% concentrations (w/w flour basis) to wheat flour to evaluate its effects on quality properties of bulky bread. Bread crumb firmness value was changed significantly with increasing of kefiran (1%, 2% and 3%) quantity compared to control. Bulky bread enriched with kefiran in the concentration of 3% expressed lower values for texture parameters. From technological parameters of the bulky bread, it has been approved that addition of kefiran in the concentration of 1% and 2% improved volume and specific volume values, while in 3% concentration, the same values presented reduction. In relation with sensory evaluation of the prepared bread samples by consumer panel experiments, the optimized values for organoleptic and sensory evaluation properties of bulky bread enriched with kefiran in 3% concentration have been detected. Finally, usage of kefiran as well as increasing of its content in dough formulation increased bulk bread stability and shelf life.

Keywords: *Kefiran; Medium Protein Wheat; Quality Parameters; Bulky Bread*

Introduction

Exopolysaccharides (EPS) are exogenous microbial metabolites mainly generated by bacteria and microalgae through their growth [1]. EPS play different roles such as protection of cell against water absorption; phagocytosis and phage invasion [1,2], however the replacements of additives in novel functional food products with lower fat contents and/or vegetarian foods were not always welcomed by markets [3,4]. An extensive range of food products have been derived from wheat and its products including breads; buns; noodles; biscuits; cakes etc. Today, there is an increasing interest for the application of edible gums as additives in baking industry to confer various functionalities to the novel foods. Several studies have been carried out expressing the potential application of hydrocolloids and microbial exopolysaccharide in the baking industries. Guarda., *et al*. [5] tested the influence of gums such as k-carrageenan; sodium alginate (Alg-Na); HPMC and xanthan gum (XG) on the physicochemical characteristics of bread, and reported that water absorption gets increased by the addition of gums, the highest absorption value was observed in HPMC application case. Friend., *et al*. [6] demonstrated that the addition of cellulosic derivative at the concentration of between 0.3 - 1% increases the shelf life of tortilla within storage process as well as resistance against cracking through rolling. Particularly the application of hydrocolloids has been found to affect the stability of dough during proofing stage. Hydrocolloids due to having higher water retention capacity increase stability of the food products that undergo successive freeze-thaw cycles through processing and storage [7]. On the other hand, pasting characterizations of wheat starch are largely modified by hydrocolloids addition, although the intensity of their impacts depends on the chemical structure; but XG and pectin increase the cooking stability, while k-carrageenan mainly affects the bump area relevant to the formation of amylose-lipid complex [8]. It should be mentioned that beside natural polysaccharides from cereal grains, microbial flora of dough used widely in sourdough fermentation process, generate a wide range of polysaccharides [9].

Aim

The aim of the present study is to examine the effect of kefiran on the fresh bread quality and its potential use in retarding the staling process during storage.

Materials and Methods

Materials

Sampling

Wheat variety Parsi cultivated extensively in Iran has been collected from two areas of Alborz province, Iran (Karaj), mixed vigorously and stored in a cool, dry and dark cabin. The initial characteristics of the sample such as protein content; Zeleny index; bread volume; moisture content; grain hardness and flour water absorption have been determined using NIR (Inframatic, 8612, Perten, Sweden), and dimension of grain; thousand kernel weight as well as hectoliter weight were determined using Caliper counter machine and hectoliter measurement device, respectively. Parsi whole grain wheat flour was obtained through Parsi wheat sample using a lab Mill (Model 279002, Brabender, Germany). The characteristics of the flour such as moisture content (method 44-16); dry gluten (method 38-10); falling number (method 56-81B); Zeleny index and SDS-sedimentation value (method 56-61A) were determined using the methods of (AACC, 2003) [10], and flour water absorption value and protein quantity have been detected using the NIR (Inframatic, 8612, Perten, Sweden). Since kefir grains (not pills) were purchased locally from market. kefiran (1, 2 and 3%); sodium chloride (Golha, Iran), commercially refined corn oil (Behpak, Iran) and instant yeast (Lesaffre, Wolczyn, Poland) were used in the dough formulations.

Methods

Starter Culture and Fermentation Medium

Fresh kefir grains used were obtained from local market (Iran-Tehran). The grains were preserved under 20^0C and reactivated using successive subcultures in fresh skimmed milk and dry milk (Golestan, Iran). Kefir grains washed carefully with sterile water were inoculated (10 gr) into 100 mL of milk. After incubation at 24^0C for 24h, the grains were separated from the fermented product by filtration through a plastic sieve (sanitized carefully by immersion in 70% ethanol solution followed by sterile water) and were washed prior to the next culture passage (subculture). Subcultures were repeated several times to increase kefir grain biomass [11].

Isolation and quantification of kefiran

Exopolysaccharide in the kefir grains were extracted by the method presented previously [12]. Briefly a weighed amount of kefir grains was treated in boiling water (1:10 w/w) for 30 min with discontinuous stirring. The mixture was centrifuged (Hanil Science Industrial, South Korea) at 10000g for 1 min at 20^0C. The polysaccharide in the supernatant was precipitated by addition of an equal volume of cold ethanol and left at 20^0C for overnight. The mixture was centrifuged at 10000g for 20 min at 4^0C. Pellets were dissolved in hot water and the precipitation procedure was repeated twice. The sediments were finally dissolved in hot distilled water (kefiran solution) [11]. The resulting solution was concentrated by the freeze dryer (Operon, South Korea) under vacuum oven (Memmert), yielding a crude polysaccharide. The samples were tested for the absence of other sugars and proteins by high-performance liquid chromatography analysis (HPLC) and phenol-sulphuric acid method [22], respectively.

Bread Making Process

The baking tests were carried out in an electric oven (Model T 6200, Heraeus, Germany). Basic dough formulation was based on 100g flour consisted of 1.5% instant yeast; 1.5% sugar; 1.5% salt; and the required volume of distilled water to reach to 500 Brabender Unit (BU) of consistency value. Kefiran was added as powder in different (1%, 2% and 3% flour basis). Firstly, instant yeast dissolved in warm water (35^0C), and further ingredients (Sugar and salt) were added, then the mixture was blended vigorously with a mixer (Model K45SS, Hobart Corporation, USA) for 3 min. Kneading was carried out manually, after kneading dough was fermented at 30^0C and relative humidity of 75% for 30 min in a fermentation chamber. The dough created was distributed into 4 parts and molded continuously. Meddle proofing process was performed at temperature of 30^{0}C at the relative humidity of 80% for 15 min. Molded dough placed in baking pans that had already been pomade with corn oil; vibrating of the pan is required avoiding the sticking of the bread to the pan within and after baking process. The bread dough had been weighed into desired sizes assuring obtaining an appropriate final loaf sizes. The weighed dough had been molded into hemisphere shape prior to being placed in a baking pan. Final proofing was performed at a temperature of 350C and relative humidity of 90% for 60 min prior baking, while baking process was performed at the temperature of 220^{0}C (428 F) for 15 min using an electric oven. The prepared breads were allowed to cool down to room temperature for 1 - 2h, then packaged into polyethylene bags and preserved at 20 ± 5^{0}C for 1 - 3 days.

Technological evaluation of bread

Physicochemical characteristics of breads including weight; volume (rapeseed displacement); specific volume index (v/m); width/height ratio of the central slice [10], and crumb texture were assessed. Crumb firmness was determined according to the AACC approved method 74-09 [10] using the Instron machine (Model TESTO 405-V1, Germany) at temperature of 20 ± 5^{0}C. Fresh bread crumb sample with the thickness of 20 mm has been used to determine the texture after 24, 48, 72h storage at ambient (20 ± 5^{0}C) conditions. An aluminum plunger with diameter equals to almost 25 mm was then released to compress (40%) to the crumb (100 mm.min^{-1}) under gravitational force of 30 N (Compression was measured up to 40% at 100 mm.min^{-1} with force of 30 N).

Sensory evaluation

Sensory evaluation of the crumb; crust appearance; brittleness; odor; taste; fracture as well as rupture and overall acceptability values of the freshly baked bulky bread samples were determined using 5 trained panelist group (3 male and 3 woman). The test was conducted after 3h of baking [5].

Data analysis

Each experimental value has been achieved in three replications and the difference between the means of each group has been detected using Duncan's multiple range test (DMRT). SPSS 16.0 statistical package (SPSS Inc., USA) was employed in the data analyses. The graphs were constructed using Microsoft Excel 2010 program (Microsoft Inc., USA).

Results and Discussion

Characteristics of Parsi grain and its flour

The achieved characteristics of the tested wheat and applied flour were shown in Tables 1 and 2.

Trait	Value
Length (mm[1])	7.46
Width (mm)	3.58
Diameter (mm)	3.18
Thousand kernel weight	42.02
hectoliter weight	77.23
Protein (%)	12.2
Bread Volume (ml[2]) (for 500 gram of dough)	527
Humidity (%)	10.56
Rigidity	51.6
Water Absorption (%)	64.36
Impurity (%)	1.1
Grain fracture (%)	0.9

Table 1: *Detected characteristics of Parsi grain.*

1: Millimeter; 2: Milliliter

Trait	Value
Moisture content (%)	10.56
Protein content (%)	11.90
Wet gluten content (gr[1])	24.40
Dry gluten content (gr)	7.20
Gluten index	19.39
Falling number (s[2])	362.33
SDS-Sedimentation height (mm[3])	60.5
Zeleny index	33.33
Flour water absorption (ml[4])	64.36

Table 2: *Detected characteristics of Parsi wheat flour.*

1: Gram; 2: Second; 3: Millimeter; 4: Milliliter

The effects of kefiran concentrations on some quality parameters are shown in Table 3 and Figure 1. Compared to control formulation, firmness was affected with the adding of kefiran. Control samples expressed the highest enhancement in hardness value after 24h of storage, and the minor influence was promoted by the adding of kefiran in the concentration of 1% in the formulation. Bread crumb containing kefiran leads to a decrease in crumb hardness; on the other hand kefiran in the concentration of 3% presented the most considerable effect on the texture of the bread, in previous investigations, the same results have been detected, that's mean, the addition of polysaccharides including HPMC; CMC; carrageenan as well as Alg causing to soft crumb of the produced wheat bread, while XG expressed the reverse result [13-15]. According to the previous results, it might be stated that origin of the polysaccharides and the methods that they have been extracted by, have the most significant impacts on the behaviours of the polysaccharides in rheological properties of the food product systems. In this respect, in the food products derived from cereals, the application of natural hydrocolloids have donated products with the most appropriate rheological properties and uniform textures. Regarding the different applied concentrations of kefiran in the formulation of dough, the significant alterations in the crumb hardness were achieved in dough with kefiran concentration of 1% w/w wheat flour basis, which the higher modifications have been obtained in further concentrations of the kefiran. Although the mechanisms of the modification impacts of hydrocolloids such as kefiran on the food products' textures have not been distinguished properly, however many studies demonstrated that hydrocolloids might diminish the structure of starch, leading to the uniform water distribution and maintenance in the texture, and as a result decline the crumb resistance [5,16,17]. Biliaderis., *et al*. [18] have proposed that the impacts of the hydrocolloids might be justified by two opposite phenomena, first an increase in the rigidity as a consequence of a decrease in the swelling of the starch granules and amylose lixiviation, and second the weakening impact on the starch structure due to an inhibition on the amylose chain associations, the intensity of the abovementioned phenomena depends on each hydrocolloids characteristics. The obtained outcomes of the constructed graphs have shown the highest and lowest significant differences between bread crumb extensibility and the area of work to peak deformation. Obtained peak values for the crumb strength have illustrated some inconsistency, which might affect the significance of the obtained test data. The test procedure determined by the elasticity and break characteristics of the samples explained generation of an indication crumb strength representing the product consistency. Therefore, bread containing of kefiran in the concentration of 3% and control showed the least and the highest consistency values, respectively.

EPS (%)	Time after of baking (h*)		
	24	48	72
0.00%	16.418^{Ca**}	56.270^{Ba}	83.220^{Aa}
1.00%	14.197^{Ca}	56.140^{Ba}	78.640^{Ab}
2.00%	10.719^{Cab}	37.742^{Bb}	55.510^{Ac}
3.00%	7.311^{Cb}	26.986^{Bc}	50.770^{Ad}

Table 3: *Effect of exopolysaccharide content on the changes in crumb softness of medium protein wheat bread by resistance to compression test (gr/N).*

**Hour*

**Different small letters in each column indicate significant difference (p ≤ 0.05). *The different capital letters in each row indicate significant difference (p ≤ 0.05).*

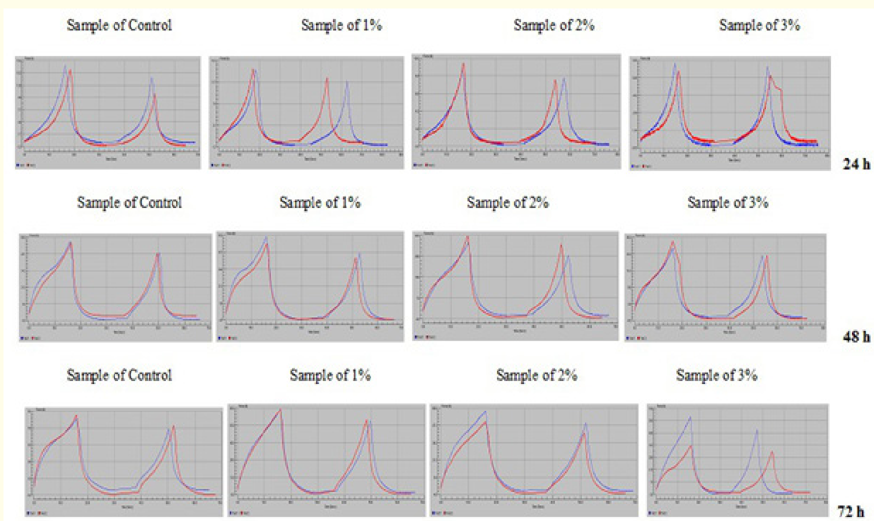

Figure 1: *Effect of exopolysaccharide content on the changes in crumb softness.*

Effect of kefiran on bread technological properties

The impacts of kefiran concentration on some qualitative parameters have been shown in Table 4. Bread volume has shown an enhancement by adding of kefiran pills, but volume was decreased at the kefiran concentration of 3%. These obtained results are in solid agreements of the previous investigations [5,13,16] that demonstrated the optimized quality of wheat bread with respect to the loaf volume. This result might be justified that kefiran addition might boost the stability of the interface dough system through proofing process and gives additional strength to the gas cells through the baking and increases the gas retention that finally leading to an optimized volume [13]. The specific volume index of the prepared bread showed a significant increase with the addition of kefiran in the concentration of up 2%, while has shown a decrease in kefiran concentration of 3% in dough formulation. The length, height and width values have been improved by addition of the kefiran in the formulation, obviously. The adding of kefiran in the concentrations of 1% and 2% led to a decrease in the width/height ratio, improving the slice shape of the baked fresh bread, however the adding of kefiran in the concentration of 3% increased the width/height value.

EPS (%)	Parameter			
	Weight (gr**)	Volume (cm^{3**})	Specific volume	Width/height ratio
0	31.36 ^{b*}	58.00 ^{d}	1.86 ^{ab}	0.437^{b}
1	33.02^{a}	61.66^{b}	1.87^{ab}	0.430^{bc}
2	33.17^{a}	66.66^{a}	2.01^{a}	0.421^{c}
3	32.30^{a}	57.66^{c}	1.82^{b}	0.493^{a}

Table 4: *Effect of kefiran on technological properties of medium protein wheat bread*

** Different letters in each column indicate significant difference ($p \leq 0.05$).*

***gr: gram, cm^3: centimeter cube*

Evaluation of bread

The achieved results of sensory admissibility of the medium protein bread samples have been expressed in Table 5. Whole of the samples were scored sufficiently from the panelists. Increasing of the applied exopolysaccharide concentrations in the formulation extremely improved the crust appearance; taste; aroma; brittleness and overall acceptability. Bread samples containing kefiran demonstrated higher moisture content and softer crumb than those without the EPS or control. With an increase in the concentration of the exopolysaccharide, the surface brittleness and dryness values were boosted. These obtained results are in a solid agreement of the previous findings of [19], that reported with adding of XG quantity in the dough formulation the surface roughness and dryness values of wheat bread were increased. The crispness of bread crust has been explained in relation of the quantity of available water; the extent of the starch gelatinization and protein modification through baking process and the water activity (aw) in the baked products [21]. Color of crust was improved with the addition of kefiran to dough formulation, whereas the obtained score of the color of the control bread's crumb was increased compared to the samples containing kefiran (exopolysaccharide).

EPS (%)	Crust appearance	Crumb structure	brittleness	Crumb softness	Aroma	Taste	Fracture and Rupture	Overall preference
0	1.50^{c*}	3.50^{a}	2.86^{c}	1.33^{b}	3.00^{b}	2.16^{c}	4.53^{a}	3.50^{c}
1	2.73^{bc}	2.16^{c}	2.93^{bc}	4.43^{a}	2.90^{b}	2.50^{bc}	4.00^{b}	3.50^{c}
2	3.23^{b}	3.00^{ab}	3.40^{b}	4.26^{a}	3.16^{b}	3.26^{b}	3.16^{c}	3.90^{b}
3	4.20^{a}	2.83^{b}	4.00^{a}	4.33^{a}	4.5^{a}	4.50^{a}	3.50^{bc}	4.60^{a}

Table 5: *Effect of kefiran concentration on the sensory acceptability of medium protein wheat bread.*

** Different letters in each column indicate significant difference ($p \leq 0.05$).*

Concerning kefiran percentage, the obtained surface fracture and rupture results presented that various concentrations of kefiran might be effective in creation of irregular crust structures. These obtaining are in solid agreements of the findings of [19,20], the mentioned scientists recorded the same results in cassava-wheat bread; gluten-free bread and French bread respectively. Shittu., *et al.* [19] reported that the presence of XG might reduce the available water sufficient for starch gelatinization due to competition with other available macromolecules in water absorption leading to surface stress and as a result irregular crust structure of the breads formulated with composite cassava-wheat. Generally, regarding the overall detected parameters as well as preference scores of the various concentrations of exopolysaccharides on the produced fresh bread quality, it could be concluded that highest acceptability might be attributed to the kefiran in the concentrations of 3%. A wide range of results could be obtained due to the processing procedure, the origin of exopolysaccharides and/or hydrocolloids.

Conclusions

Kefiran as a high molecular weight polysaccharide obtained from Iranian kefir grains improved some rheological properties of the dough. Addition of kefiran revealed improvements in bread quality such as loaf volume, crumb softness, percentage of cell area and overall sensory acceptability. It is revealed that to achieve to an optimized formulation in bakery products rheological properties alongside sensory characteristics should be detected. However as has been found kefiran started alterations in texture properties of bakery products from 1% concentration. kefiran and its polysaccharides could be introduced as a compound that could make optimized alterations in physico-mechanical properties of the bakery products, beside this, it should be noticed that this compound has been considered with bioactivity making this product for application in a wide range of industries such as nutraceutical, pharmaceutical and cosmetic.

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