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Hydrocolloids-Germinated Wheat Flour Interactions in the Formulation of Lavash Bread Packaged with an Active Modified Atmosphere Packaging System

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ABSTRACT: A modified formulation and an efficient packaging system have improved flatbreads with short storability, resulting in long shelf life and exportability. This research investigates the use of germinated wheat powder, κ-carrageenan, and guar gum in a modified atmosphere packaging system to enhance the storability of Lavash, an Iranian flatbread. A three-component D-optimal mixture design was used to create sixteen dough formulations, determining the best ones based on rheological and thermal analysis data. The Mixolab test was used to analyze retrogradation rates. The optimal formulation demonstrated a synergistic effect between κ-carrageenan (0.055%) and germinated wheat powder (0.208%), increasing moisture content up to 9% and decreasing setback factor to 28%. Principle component analysis (PCA) was used to analyze the correlation between the rheological, textural, and sensory attributes of dough and baked bread samples, influenced by the type and quantity of hydrocolloids. Chewability was highly correlated with Principal component analysis No. 1 and adversely affected by guar gum and carrageenan, while the setback factor was correlated with Principal component analysis No. 2 and negatively affected by malt. Thermoformed polyvinylchloride containers with water and oxygen absorbent sachets were used as active modified atmosphere packaging (Active-MAP) to preserve microbial and textural characteristics for up to 2 months at 30 °C.

Keywords: Active-MAP, Flatbread, Germinated Wheat Powder, Guar Gum, κ-carrageenan.

Introduction

Flatbreads are one of the most popular traditional bread types in several parts of the world. The global flatbread market is predicted to increase at a CAGR of 6.2 percent, from $38.8 billion in 2018 to $62.8 billion, between 2019 to 2026. (Business wire, 2020). Iran stands as the second greatest consumer of bread worldwide. By 2022, the mean bread consumption per household had increased to 161 kilograms, from approximately 150 kilograms in 2011. The rate of consumption is two to three times that of other countries (World Grain, 2023). "Lavash" is a fermented single-layered flatbread with an elliptical shape and a thickness of 0.3–0.5 cm, made with 2/3 wheat flour, 1/3 water, salt, and yeast (Qarooni, 1996; Kumar, 2016). Flatbreads were once baked manually in low-cost traditional ovens, but modern lifestyles

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and conscious consumers' expectations demand a large-scale industrial product with high quality, minimal waste, and long shelf life. Lavash may not gelatinize as much as other bread types because it is baked at high temperatures for a short time. Despite its soft texture, the moisture in this bread quickly evaporates, leaving it dry and stale (Salehifar *et al*., 2009). Hydrocolloids are a large group of polysaccharides that are commonly utilized in bakery products to improve texture and retard staling (Ghoreyshirad *et al*., 2011; Fadda *et al*., 2014). Carrageenans are a family of natural polysaccharides found in edible red seaweed that interact with proteins to form complexes with improved microstructural and rheological properties (Seo & Yoo, 2020). κ-carrageenan interacts positively with gliadin and enhances water retention in the dough while lowering stiffness in the bread texture (Leon *et al*., 2000; Rosell *et al*., 2001). Ghanbari & Farmani (2013) used κ-carrageenan to improve the overall acceptability of a traditional flatbread. Hongsprabhas *et al*. (2007) also found that a hydrocolloid effects on retrogradation, gelation, and setback factor depend on a specific starch–hydrocolloid combination. According to Funami *et al*. (2008), hydrocolloids accelerated short-term retrogradation (amylose-amylose aggregation) and slowed long-term retrogradation (related to amylopectin-amylopectin associations).

Another group of anti-stalling agents includes α-amylase from different sources. The commercial fungal amylases produced by *Aspergillus Oryzae* are suspected to be linked to the risk of developing allergic respiratory disease (Hopek *et al*., 2006). Sprouted (germinated) wheat flour is another source of this enzyme, containing soluble proteins, minerals, proteases, xylanases, and flavor compounds, and could be used as an alternative to commercial flour improvers (Majzoobi *et al*., 2012; Marti *et al*., 2017). Using this nutritive additive, with enzymatic activity in bread dough formulation, at a level of 0.2-0.5%, may provide optimal yeast growth, better moisture retention, and improved rheological properties in the dough and the final product (Pyler & Gordon, 1988; Hrušková *et al*., 2003). According to the Code of Federal Regulations, this additive can be mixed with wheat flour to compensate for any natural deficiency of enzymes (FDA, 2019). For industrial Lavash, wheat flour with a falling number of 300 seconds is usually used to provide the necessary degree of α-amylase activity (Feyzipour *et al*., 2004). Rosell *et al*. (2001) studied the effects of a combination of different anti-stalling agents on the characteristics of wheat dough and observed a synergistic effect between α-amylase and κ-carrageenan. On the other hand, there has been evidence of a negative interaction between this amylolytic enzyme and guar gum (the commonly consumed hydrocolloid in the formulation of industrial Lavash), resulting in the formation of an inactive enzyme-gum complex (Brennan *et al*., 1995; Slaughter *et al*., 2002; Dhital *et al*., 2015). This latter point was one of the most important considerations in the current research, which intends to develop a dough formulation with alternative hydrocolloids for guar gum.

Another practical way to prolong the shelf life of industrial bread is to benefit from an efficient packaging system. Active MAP is a type of MAP. The term "active" emphasizes the role of food preservatives in addition to the simple MAP technology (Charles *et al*., 2006). One main issue with bakery products is the incomplete elimination of oxygen by the

MAP system, which may lead to mold growth even at low levels of residual oxygen during extended storage at room temperature. Moisture entrapment in the package may be considered as another spoilage factor because it accelerates microbial growth. Both of the aforementioned issues can be avoided by incorporating absorbent sachets into the packaged food product (Ooraikul & Stiles, 1991).

The main objectives of this research were to develop a new dough formulation for Lavash by using a combination of anti-stalling additives along with the active modified atmosphere packaging system in order to obtain the following results:

Investigating whether three hydrocolloids—guar gum, κ-carrageenan, and germinated wheat powder—interact in a novel formulation of a popular thin flatbread. Determination of the optimal dough formulation based on the thermal, rheological, and staling properties. The safety aspects of the newly developed formulation were investigated, taking into account the highest amount of κ-carrageenan that can be consumed daily. Finally, the long-term storability of this highly-consumed flatbread was investigated by using modified atmosphere packaging under various conditions for up to 60 days at 30 °C.

Materials and Methods

- *Materials*

The wheat flour and germinated (sprouted) wheat powder (GWP) were provided by Alborz Flour Mill Co. (Karaj, Iran) and Shahd Zagros Jahanbin Co. (Chaharmahal and Bakhtiari provinces, Iran), respectively. Guar gum (FCC/food Grade/E412, CAS No: 9000-30-0) was obtained from RP International Limited (Shanghai, China) and κ-carrageenan (viscosity: > 20 cps. RT, pH: 6, protein: 0.2 g/100g, total count< 5000CFU/g) was obtained from GPI, Inc. (Ontario, Canada). The packaging materials provided by Plastic Alvan Machine Co. were as follows: (1) polyethylene terephthalate pouches (laminated cast polyethylene terephthalate/linear low-density polyethylene film) (2) thermoformed containers made from polyvinyl chloride/linear low-density polyethylene sheets with polyester lids. Ferrous iron powder sachets (150cc, Raz Salamat Paya Co. (Tehran, Iran)) and granular silica gel sachets (1gr, Motlagh Scientific Research Group, Tehran, Iran) were used as the oxygen and moisture absorbers, respectively.

- *Methods*

- *Raw* ***materials specifications***

The physicochemical properties of wheat flour were analyzed according to AACC (2021). Different mixtures of (0.1-0.6%) GWP and wheat flour were prepared to determine the appropriate amount of GWP in the dough formulation, and the α-amylase activity in the mixtures was measured using the Falling number test (Bastak, 5100; Moris Technology; Lithuania).

- ***Bread preparation procedure***

The low-salt Lavash dough formulation consisted of 100kg wheat flour, 50kg water (based on flour, as determined by the Farinograph test, (AACC, 2000)), 0.5% salt, 0.23% yeast, 1.5% Guar gum, and 5 ppm fungal α-amylase (Fungamyl 4000SG (Novozymes, A/S, Denmark)) was prepared as control. All of the dry ingredients were weighed and added to the flour and water in a dough mixer (Escher MR industrial spiral mixer, Italy) and then combined for 10 min at 20 rpm. The prepared dough was allowed to rest (fermentation stage) at 30˚C for 80 min before being shaped into a sheet with a thickness of 1mm using an in-line sheeter apparatus (BVT: Dough Process Solutions, OSS, Netherlands) and baked in a tunnel oven at 120˚C for 20 sec. After cooling to 30˚C, the sheet was cut, stacked, and transferred for packaging.

- *Experimental Design*

The composite Lavash dough formulations consisted of three ingredients were designed by the Design-Expert software (Sat-Ease ver. 13). A D-optimal mixture design was used with the constraints of GWP (A), κ-carrageenan (B), and guar gum (C). The components levels were considered in the range of 0-0.3%, which was set after preliminary trials and literature surveys. Sixteen experimental treatments, including 6 model terms, 5 replicates, and 5 lack of fit points, were designed (Table 1).

- *Analytical methods of Lavash dough formulations*

The Farinograph, Extensograph, and Amylograph tests (AACC, 2000) were used to assess ten different quality parameters of the designed dough samples, including water absorption (%), dough development time (min), dough stability (min), extension resistance (BU), maximum resistance (BU), extensibility (mm), energy, pasting temperature (˚C), peak temperature (˚C), and peak viscosity (AU). The data acquired for each response was fitted to statistical models to evaluate the regression coefficients and study the interactions between the constituents. The results of the Farinograph, Extensograph, and Amylograph tests were then utilized to choose dough formulations based on the desired goals for the following responses, predetermined for the thin flatbread dough follows (Wrigley, Batey & Miskelly, 2017): medium dough development time (min), maximum extension resistance (BU), maximum extensibility (mm), and maximum peak viscosity (AU).

Table 1. Dogh Samples Formulations

Sample	Germinated Wheat Powder (%)	κ - Carrageenan (%)	Guar gum (%)
1	0.150	0.000	0.150
2	0.000	0.300	0.000
3	0.150	0.150	0.000
4	0.000	0.150	0.150
5	0.000	0.300	0.000
6	0.000	0.000	0.300
7	0.125	0.125	0.050
8	0.000	0.150	0.150
9	0.050	0.200	0.050
10	0.000	0.000	0.300
11	0.150	0.000	0.150
12	0.300	0.000	0.000
13	0.050	0.050	0.200
14	0.100	0.100	0.100
15	0.200	0.050	0.050
16	0.300	0.000	0.000
Control	0.000	0.000	0.000

Finally, using the Mixolab test (Chopin Technologies, France) based on the following protocol, the retrogradation rates of the previously selected formulations were compared to control to determine the best dough formulation: mixing speed: 75 rpm; tank temperature: 30° C; first, second and third plateau temperatures: 30, 90, 50 °C; first, second and third plateau durations: 20,7 and 5 min; heating and cooling rate: 4° C/min; total analysis time: 57min. (Rosell *et al*., 2007).

- *Bread samples production and packaging*

Based on optimal and control formulations, the bread samples were industrially produced in a continuous production line as described earlier and then packaged in flexible polyethylene terephthalate (PET) pouches as the commercial bread packaging materials. A mixture of 80% CO_2 and 20% N_2 was flushed into the PET pouches using the Henco-Vac Machine (T3, The Netherlands). All samples were kept at T=29±1°C and RH=40% and analyzed after 0, 10, 20, 30, and 60 days of storage. Microbiological tests of the bread samples were conducted according to ISIRI (2015) to study mold, yeast, and coliform contamination after each term of storage. An alternative packaging strategy has been further applied by using thermoformed containers to extend the microbiological shelf life and provide better exportability. The same gas mixture was flushed into the containers utilizing the Roll-fed thermoforming machine for MAP packages (R575 MF; Multivac, Germany). These packages were divided into two groups: (1) gas-only (normal MAP) and (2) gas-plus-oxygen-and-moisture-absorbing sachets (active MAP). The packages were kept at the same storage conditions and analyzed after 0, 10, 20, 30, and 60 days of storage.

- Analytical methods of the bread samples

- The bread samples were subjected to microbial analysis, including total coliforms, yeast, and mold (ISIRI guidelines, 2015).
- The infra-red Sartorius moisture analyzer (MA 35, Gottingen, Germany) and the Lab master water activity meter (Novosina, Switzerland) were used to determine the moisture content (AACC, 2000) and water activity of samples, respectively.
- The textural characteristics of the bread samples were investigated by a Warner-Bratzler test (AACC, 2000) using the TA-XT Plus texture analyzer (Stable Microsystems Ltd., UK.) with a straight blade (code no. HDP/BS) as follows: bread samples were first cut into 5cm × 7 cm rectangular pieces and then three pieces were sheared together according to the method of Shah *et al*. (2017).
- An OXYBABY® mobile gas analyzer was used to control fluctuations in the gas composition (% O_2, % CO_2, and % N_2) in the packages of the bread samples during storage at 30 C. (WITT Gasetechnik GmbH & Co.KG).

- Statistical Analysis

The results were subjected to analysis of variance with a confidence level of 95%, and appropriate mean separation was conducted using Duncan's multiple range test in SPSS software (IBM ver.22).

Results and Discussion

- Physicochemical properties of wheat flour and the amylolytic activities of wheat flour and GWP mixtures

Table 2 indicates the physicochemical parameters of the original wheat flour. All of the properties (except falling number) are within the range of a weak to medium quality of flour that is suitable for a single-layered flatbread such as Lavash (AACC, 2021; Tavakolipour *et al*, 2006; Kamaliroosta *et al.,* 2016). The amylolytic activities of various GWP and original flour combinations are shown in Table 3. This information helps in determining the amount of GWP required to produce a thin flatbread dough with sufficient amylase activity. According to the data, 0.3% of this ingredient may provide enough enzymatic activity (Falling number = 292 sec) for dough formulation. Generally, a falling number between 250 and 300 seconds indicates that the enzyme activity in flour is well-balanced (Feyzipour *et al*,. 2004). If this parameter is equal to or more than 400s, it will denote low enzyme activity, which should be compensated for using commercial fungal amylases (Ćurić *et al*., 2002). These enzymes have been used to improve the quality of wheat flour for a long time and are still one of the most popular additives flours (Hayden 1961; Pritchard 1992; Elyasifar *et al*. 2020). Germinated wheat flour is another alternative for providing α –amylase activity with some advantages compared to microbial enzymes that will be discussed later (FDA, 2019, Dhillon *et al*. 2020).

Table 2. Physicochemical properties of the original wheat Flour

Physicochemical properties	Values [a]
Moisture (%)	13.40±1.10
Ash (%)	1.10±0.06
pH	5.43±0.09
Lipid (%)	1.2±0.08
Protein (%)	12.06±0.02
Wet Gluten (%)	28.20±0.20
Gluten Index	82.50±20.40
Sedimentation Value (ml)	19.47±1.23
Falling Number (sec)	476.66±28.01

[a] The results are expressed as mean ± standard deviation.

Table 3. α- amylase activity in the mixtures of wheat flour with different amounts of GWP

GWP (%)	Falling No.(s)[a,b]
0	359.0±2.6
0.1	325.0±2.6
0.2	311.7±8.3
0.3	292.5±8.2
0.4	307.3±16.3
0.5	265.5±5.3
0.6	271.0±5.2

[a] These results are expressed as the mean ± standard deviation

[b] The α-amylase activity in 3 and 6 ppm of the commercial fungal enzyme were 345s and 330s, respectively

- *Rheological and thermal properties of Lavash dough formulations and the statistical models*

Table 4 provides the rheological and thermal properties of the control and sixteen statistically designed dough samples, while Table 5 presents the fitted statistical models and regression coefficients to interpret the contribution of ingredients to each response parameter.

The linear model was suggested for water absorption with high R2 values (0.90). While the dough stability, extension resistance, extensibility, energy, pasting temperature, and peak temperature were well-fitted to the "special cubic" model (R2 =0.872-0.997). On the other hand, the peak viscosity was the only factor fitted to the quadratic model (R2 =0.96). 3D-surface graphs of water absorption, extensibility, and peak viscosity are presented in Figure 1a, b.

All of the three components, including geminated wheat powder (GWP), κ-carrageenan, and guar gum, had positive coefficients, confirming their positive contribution to water absorption (Figure 1a). Previous studies have reported that fungal α- amylase may reduce the water absorption of wheat flour, while the enzyme from malt flour can increase it (Sanz Penella 2008; Boz *et al*. 2010).

This positive effect could be attributed to the chemical components of germinated wheat flour, such as protein and crude fiber, which have been shown to increase the water absorption capacity (Dhillon *et al*., 2020). In our research, the GWP consisted of 7.68% protein and 1.54% crude fiber (according to specification data provided by Shahd Zagros Jahanbin Co.) that may have caused the positive contribution to the water absorption parameter. Furthermore, hydrocolloids (κ-carrageenan and guar gum) may promote water absorption by creating stronger bonds with water molecules via hydroxyl groups (Guarda *et al*., 2004). Interactions between hydrocolloids such as κ-carrageenan and starch have been considered as the affecting parameter in the dough of flatbread (Pahwa *et al*. 2016). A positive effect was observed on dough development time and stability time by adding each component to the dough formulation with high R2 values (0.93 and 0.99, respectively). The same result has been previously reported for both guar gum and κ-carrageenan when added solely to the formulation of an Indian flatbread, **Chapatti** (Ghodke Shalini *et al* 2007). Even though the two-component interactions on stability time as a flour strength index are negative, the three-component interactions are positive, and this means that the presence of all three additives in the dough formulation may result in a stronger dough. A three-component interaction was also revealed for one of the crucial rheological parameters of the Lavash dough, i.e., extensibility (Figure1b). It means that the interaction of three ingredients, GWP, carrageenan, and guar gum, has a significant positive and negative impact on extension resistance and extensibility, respectively. Since Lavash is a type of thin, single-layered flatbread, maximum extensibility is preferable. According to the results, germinated wheat powder combined with κ-carrageenan had the highest two-component coefficient for dough extensibility ($\beta 12 = 94.45$), whereas the addition of guar gum to this mixture changed it, oppositely ($\beta 123 = -623.4$). It seems that guar gum may interfere with the beneficial interaction between GWP and κ-carrageenan. Murayama *et al*. (1995) studied the rheological properties of mixed gels consisting of κ-carrageenan and different galactomannans such as guar gum and indicated that these two hydrocolloids could not interact successfully because of the low ratio of the main chain to the side chain in the guar gum structure, which did not provide enough free zone for binding of carrageenan.

Guar gum, on the other hand, seems to be incompatible with geminated wheat flour. This result may be due to the inhibitory effect of guar gum on α-amylase activity (Slaughter *et al*., 2002). Some of the other quality parameters showed the same interfering pattern, which fit well with a "special cubic" model.

The selected dough formulations were statistically established based on the required range of properties declared in the experimental design section (Table 6) after studying the models and interactions between the dough constituents. Three dough formulations were suggested, with guar gum concentrations of zero in two and 0.037 percent in the third. This is a far lower concentration of guar gum than is typically used in Lavash dough (i.e., 1.5%). Thus, one of our main objectives, which was to reduce the level of guar gum in the Lavash dough formulation due to its negative effect on α- amylase activity, was met.

- ***Retrogradation index (Setback factor) for the statistically suggested dough formulations***

Staling retardation is another critical parameter that has been considered to provide a long shelf life and export feasibility. Table 6 shows some of the results of Mixolab testing for the selected formulated dough samples and the control. Only the data corresponding to the starch characteristics are given in Table 6: C3 (starch gelatinization), C4 (amylolytic activity), C5 (starch retrogradation), and setback/retrogradation factor (the torque difference (C5-C4) during the cooling period) (Rosell *et al*. 2007; BeMiller *et al*. 2011). All of the suggested formulations showed a lower setback as an index of retarded retrogradation as compared to the

Table 4. Rheological and thermal properties of the formulated dough samples

Sample	Farinograph			Extensograph				Amylograph		
	Water absorption (present)	**Development Time (min)**	**Stability (min)**	**Extension Resistance (BU)**	**Maximum Resistance (BU)**	**Extensibility (ml)**	**Energy (Cm^2)**	**Peak Temperature (°C)**	**Pasting Temperature (°C)**	**Peak Viscosity (AU)**
1	55.8	5.5	6.7	398	414	113	64	84.3	61.4	683
2	56.7	5.7	7.3	633	635	93	78	90.5	62.6	1516
3	56.3	4.7	6.2	360	371	124	65	84.1	62.1	668
4	56	5	6.6	326	332	110	53	89.4	61.9	1507
5	56.7	5.7	7.3	633	635	93	78	90.5	62.6	1516
6	56.1	6.2	6.8	633	654	102	87	89.9	73.4	1481
7	55.9	5.3	7.5	574	594	98	77	87.1	62.9	777
8	56	5	6.6	326	332	110	53	89.4	61.9	1507
9	56.4	5.2	7	680	695	94	85	88.1	62.1	1038
10	56.1	6.2	6.8	633	654	102	87	89.9	73.4	1481
11	55.8	5.5	6.7	398	414	113	64	84.3	61.4	683
12	56.2	4.5	7.1	680	720	102	93	83.6	66.1	442
13	56.5	6.5	7.4	555	580	106	80	88.6	62.3	1181
14	56.3	5	7	531	550	102	76	88.6	62.4	1180
15	56.5	5.5	6.7	538	587	108	83	86.6	62.1	692
16	56.2	4.5	7.1	680	720	102	93	83.6	66.1	442
Control	56.2	5	6.6	538	567	108	77	89.6	62.4	1436

Table 5. Regression coefficients and correlations for the adjusted model to experimental data in D-optimal mixture design for the responses

Variable	Model	Coefficients							R^2
		β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}	
Water Absorption	Linear	56.0867	56.6086	55.9436					0.9032
Dough Development Time	Linear	4.61627	5.3799	6.13457					0.9314
Dough Stability	Special Cubic	7.06013	7.30113	6.82519	-3.91367**	-0.989247**	-1.78725**	28.057**	0.9986
Extension Resistance	Special Cubic	676.314	634.664	635.801	-1188.84**	-1036.7**	-1220**	9589.26**	0.9952
Maximum Resistance	Special Cubic	715.056	645.731	653.089	-1222.27**	-1094.21**	-1230.86**	9912.3**	0.9980
Extensibility	Special Cubic	102.316	91.8165	102.681	94.4525**	46.6295*	49.6295*	-622.385**	0.8721
Energy	Special Cubic	92.8097	79.4097	87.3258	-78.8389*	-103.314**	-114.114**	787.896**	0.9032
Peak Temperature	Special Cubic	83.8267	90.3517	89.8508	-11.5436*	-9.42368*	-3.57368	90.8237**	0.9697
Pasting Temperature	Special Cubic	66.1025	62.7525	73.0841	-7.11125	-34.1228**	-24.8228**	54.9779*	0.9707
Peak Viscosity	Quadratic	451.593	1482.59	1471.68	-1008.85*	-887.266*	174.734		0.9600

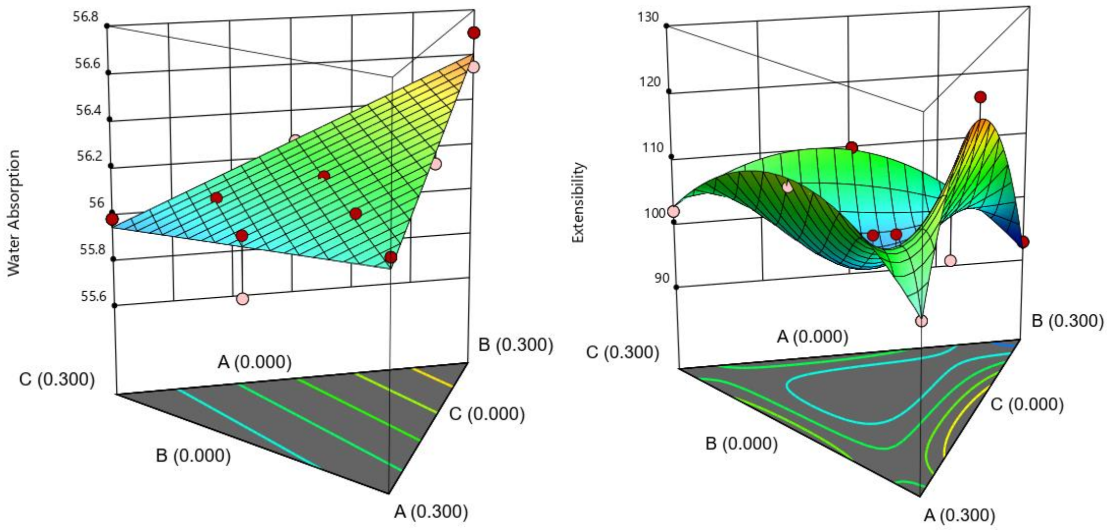

Fig. 1. Effects of the three components of A, B, and C on dough properties (a) water absorption (b) extensibility A= Germinated wheat flour, B= κ-carrageenan, C=Guar Gum

Table 6. The statistically selected dough formulations and the Mixolab parameters

Samples	Components			Mixolab Parameters			
	Germinated Wheat Powder (%)	κ -Carrageenan (%)	Guar Gum (%)	C3 (Nm)	C4 (Nm)	C5 (Nm)	Set Back (Nm)
Control	0.000	0.000	0.000	1.908	1.847	3.631	1.723
Selected 1	0.245	0.055	0.000	1.786	1.742	2.975	1.189
Selected 2	0.208	0.055	0.037	1.775	1.696	2.912	1.137
Selected 3	0.150	0.150	0.000	1.774	1.733	2.999	1.225

control. The amylolytic action has been previously shown to be effective in lowering this index (Codina *et al*. 2019). In addition, the effects of different hydrocolloids added to dough formulations were inconsistent (Rosell *et al*. 2007; BeMiller *et al*. 2011; Vazquez & Veira, 2015). However, in the current research, a combination of the three parameters (one enzyme source and two types of hydrocolloids) has been investigated; and it was concluded that the second suggested formulation with the lowest setback (C5-C4=1.137Nm), containing 0.055% κ-carrageenan, 0.208% GWP, and 0.037% guar gum, may be introduced as the best one. The statistically predicted rheological and thermal properties for this best formulation were as follows: water absorption (56.193%), dough development time (4.96min), dough stability (6.896 min), extensibility (107.8 mm), maximum resistance (577.68 BU), peak temperature (84.837 ºC), and maximum viscosity (566.427 AU). The desirable thermal/rheological characteristics and also the lowest retardation rate of the optimal formulation may confirm the perfect cooperation between κ-carrageenan and guar gum. It is well-known that certain galactomannans and particular helix-forming galactans from the carrageenan family have a synergistic impact (Viebke, 1995).

- ***Evaluation of the best bread formulation from the consumers' safety aspect***

Another key aspect of this research was comparing the κ-carrageenan content of the best dough formulation to the maximum permissible level of k-carrageenan, based on daily flatbread consumption. According to Weiner (2014), no adverse effects are expected in humans (infants and adults) consuming 0.1-5 percent carrageenan per day from food products. The average daily bread intake in Iran, including Lavash, is 300 grams per person per day (Fars News Agency, 2019), and based on the minimum authorized level of carrageenan, up to 0.3 percent of this additive can be used in the daily diet. A hundred grams of wheat flour yields 110 grams of Lavash in the industrial setting, which equates to 0.0499 grams of κ-carrageenan per 100 grams of the best-formulated Lavash or 0.150 grams each day. As a result, this composition is absolutely safe to introduce to industrial flatbread producers.

- ***Analysis of the packaged bread sample with the best formulation during storage***
- ***Microbial analysis***

Microbial analysis was performed on the bread samples manufactured with the best formulation and wrapped in flexible PET pouches during storage (Table 7). The ambient air in the pouches, as expected, could only preserve bread samples for a few days (less than 10 days). The bread samples packaged in PET pouches under modified atmospheric conditions (Control + MAP/PET pouches) had the same unsatisfactory result. While the samples packaged in the thick wall-thermoformed containers under MAP and active-MAP conditions had a long microbial shelf life of up to 30 days and 60 days, respectively (Table 7).

- ***Moisture content and Water activity***

Figure 2 depicts the combined pattern of changes in the moisture content and water activities of bread samples over time. The moisture content of all of the best dough- created bread samples with varying packaging conditions is much greater than the controls, as seen in this graph (24.3-24.7 % vs. 22.3-23.7 %). It seems that the moisture content of bread samples is influenced by the presence of κ-carrageenan in the best formulation and its synergistic effect with GWP (i.e., α -amylase). According to Farinograph test data, all samples containing varied quantities of k -carrageenan (codes: 2, 3, 5, 9, 13, 14, and 15) showed higher water absorption than the control (56.2 %). The sample formulated with the highest concentration of κ-carrageenan (0.3%) had the highest water absorption (56.7 %). The water retention ability of this ingredient is revealed by variations in moisture content throughout the storage of bread samples. After 30 and 60 days of storage, the best-formulated ones with modified environment packing, with and without absorbents, showed the least moisture loss. The synergy of different carrageenan types in starch-based food products such as bread was previously described (Bemiller, 2019) and may be exploited to improve product quality by retaining moisture.

Besides, this effect of κ-carrageenan resulted in minimal changes in the water activity of bread samples during storage. The active-MAP bread samples produced from the best dough formulation exhibited no significant change in aw after 60 days of storage due to the use of moisture absorber sachets in the packages. In contrast, the regular MAP bread samples with control and the best dough formulation showed the most significant increase in this parameter, which was undesirable (Figure2). The positive role of the moisture absorber sachet in restricting moisture vapor accumulation inside the package, along with effective regulation of moisture release from bread texture by carrageenan, may prevent mold growth in the best formulation samples with active-MAP (Gaikwad *et al.*, 2018). On the other hand, a high relative humidity level in the air inside the packaging may have a negative impact on the textural characteristics of this thin type of flatbread, resulting in an undesired soft texture.

- *Textural properties*

The shear force and shear energy of the packaged bread samples were also studied as textural parameters (Figure 3 a, b). According to the results, the shear force of all samples, with the exception of the best formulation bread with active MAP, significantly changed up to 60 days of storage. Therefore, the only condition that could protect the freshness of thin flatbread and increase its shelf life to 60 days compared to 10 days for the control was the active MAP with the best formulation in thermoformed containers.

Table 7. Microbial analysis of bread samples with the best (B) and the control (C) dough formulations packaged under air, MAP and active-MAP medium in either PET pouches or thermoformed containers [a]

Samples [b]	Coliform [c]					Molds and Yeasts [d]				
	0	10	20	30	60	0	10	20	30	60
C + PET Pouches+ air packed	$<L_1$	--	--	--	--	$<L_2$	--	--	--	--
C + PET Pouches +MAP	$<L_1$	--	--	--	--	$<L_2$	--	--	--	--
C + Thermo. Cont.+ MAP	$<L_1$	$<L_1$	$<L_1$	$<L_1$	--	$< L_2$	$< L_2$	$< L_2$	--	--
C +Thermo Cont.+A-MAP	$<L_1$	$<L_1$	$<L_1$	$<L_1$	--	$< L_2$	$< L_2$	$< L_2$	$< L_2$	--
B + PET Pouches+ air packed	$<L_1$	--	--	--	--	$< L_2$	--	--	--	--
B + PET Pouches +MAP	$<L_1$	--	--	--	--	$<L_2$	--	--	--	--
B+ Thermo. Cont.+ MAP	$<L_1$	$<L_1$	$<L_1$	$<L_1$	--	$< L_2$	$< L_2$	$< L_2$	$< L_2$	--
B+ Thermo.Cont.+A-MAP	$<L_1$	$<L_1$	$< L_1$	$<L_1$	$<L_1$	$< L_2$	$< L_2$	$< L_2$	$< L_2$	$< L_2$

[a] L_1 = Coliforms Acceptance Limit: 10Cfu/g and L_2 = Molds and Yeasts Acceptance Limit: 10^2CFU/g
[b] Thermo.Cont. = Thermoformed Container; A-MAP = Active MAP

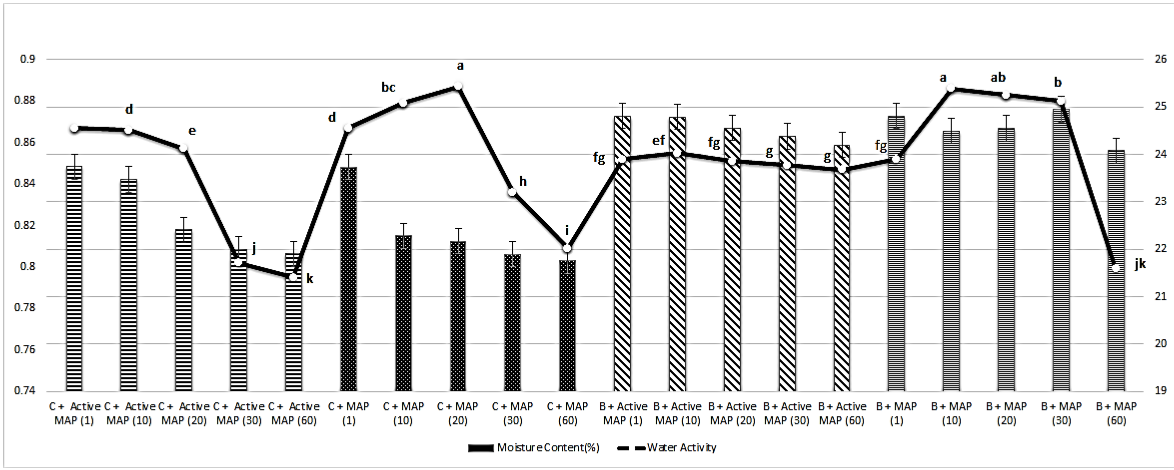

Fig. 2. Changes in moisture content and water activity of the best- formulated (B) and the control (C) bread samples packaged under air, MAP and active-MAP medium during storage

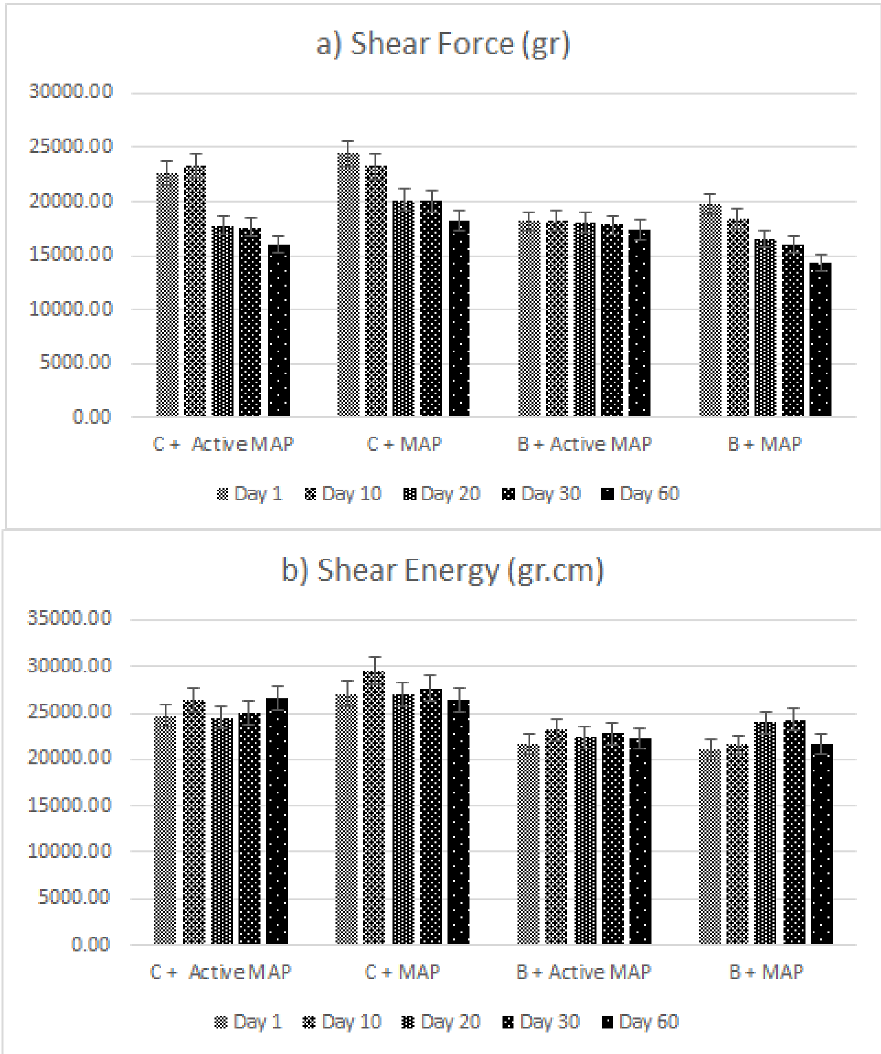

Fig. 3. Changes in shear force (a) and shear energy (b) of the bread samples with the best (B) and the control C) dough formulations packaged under air, MAP and active-MAP medium during storage.

- Headspace gas concentration

The headspace gas mixture used in the MAP system, which may alter due to penetration through the package while stored at 30°C, was also studied in this study (Figure 4). Even though the barrier characteristics and thickness of the sheet (PVC/LLDPE, 540/60) used for bread packaging trays in active MAP samples were appropriate, the relatively high storage temperature (30°C) may somewhat accelerate the oxygen permeation from storage environment into the package (Blackistone, 1999). Thus, placing an oxygen scavenger sachet in the active MAP system is a safe tool to provide an extended/mold-free shelf life for bread samples (Kotsianis *et al*., 2002; Muižniece-Brasava *et al*., 2012). The oxygen scavenger utilized in the active MAP samples creates an oxygen-free environment inside the packages, whereas in the regular MAP samples without the absorber, the level of this gas increased by up to 2%. The carbon dioxide level was also reduced by 16.5-20% compared to its initial amount in the gas combination. Because the CO_2 permeability of rigid PVC is approximately three times that of its O_2 permeability (Arvanitoyannis, 2012), a reduction in carbon dioxide gas concentration inside the package was expected. However, based on the results of the microbiological study, it can be inferred that, while CO_2 permeated out of the package, the amount of CO_2 that remained in the package for up to 60 days was still sufficient to prevent mold growth.

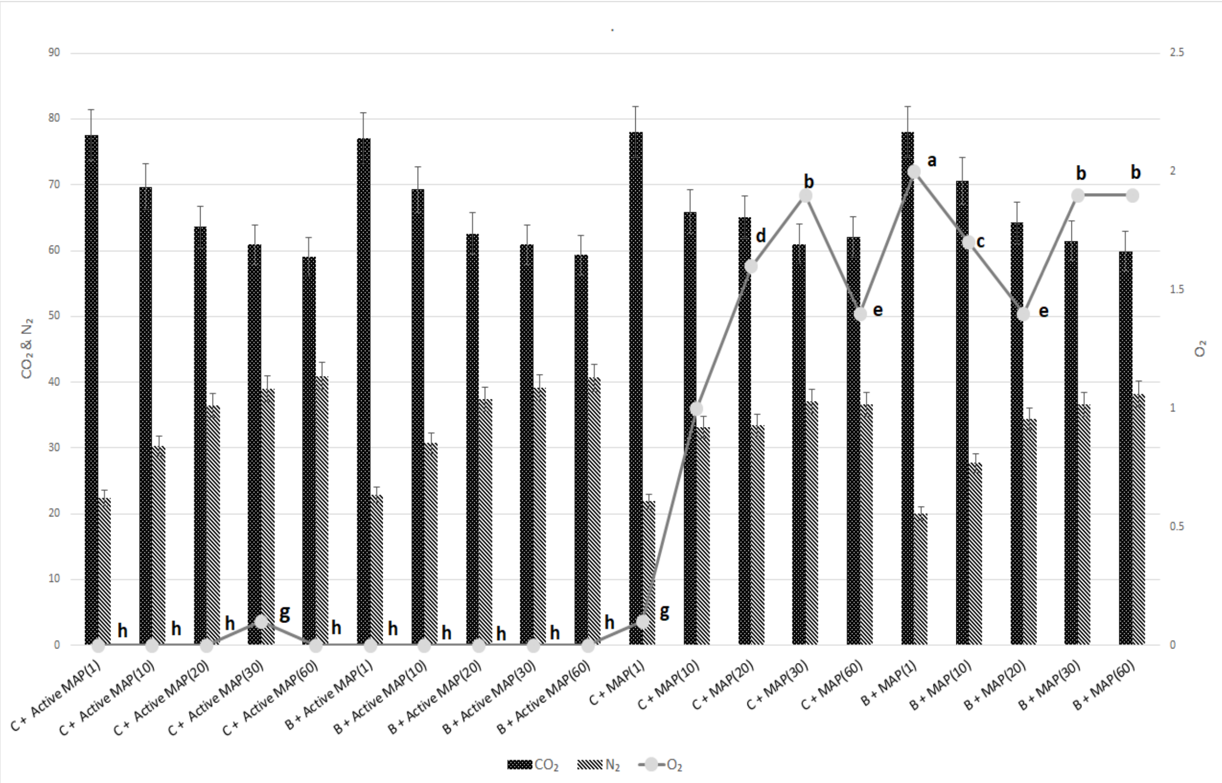

Fig. 4. Changes in the headspace gas mixture during storage at 30°C

Conclusion

Nowadays, providing a long shelf life for a food staple is a necessity worldwide, and this can only be achieved if multiple tools are used simultaneously. Using a combination of anti-staling agents and active MAP may successfully preserve a thin flatbread with a high level of waste due to rapid staling and mold growth, such as Lavash. κ-carrageenan (0.055%) showed the best synergistic effect with 0.208% GWP and 0.037% guar gum in improving water retention and retarding retrogradation of bread samples packaged in PVC/LLDPE trays by an active MAP system. Since these types of plastic containers are sufficiently freeze-resistant, future research might focus on the freeze/thaw stability of the packaged-best formulated frozen bread samples.

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