Effect of Okra (*Abelmoschus esculentus* L.) Mucilage on the Rheological Properties and Water Activity of Rice Bread

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ABSTRACT

This work was designed to study the effect of carboxymethyl cellulose **(CMC)** as synthetic hydrocolloid agent and okra mucilage (OM) as a natural one on the rheological properties of rice bread (RB) compared with regular wheat bread (WB). The water activity **(a_w)** of the prepared bread samples was also determined. Therefore, texture profile analysis (TPA) was employed using CT3 Texture Analyzer and Decagon Aqualab Meter Series 3TE for a_w. The results revealed cohesiveness, resilience, and springiness of fresh WB (T1) are higher than those of RB (T2), which are decreased by extending the storage period. On the other hand, the hardness, chewiness, and gumminess of fresh WB (T1) are lower than those of RB (T2). These characteristics are generally decreased in the fresh RB by increasing the ratio of added hydro-colloidal agents (CMC or OM). The best results were obtained by the addition of 2.0g CMC (T6) or 3.0g OM/100g (T14) to the RB formula. The water activity a_w of the fresh WB (T1) is 0.898 which gradually decreased reaching to 0.884 with no significant difference. The same trend was also observed for RB (T2). On the opposite as the hydrocolloid agent increased in the RB formulation the a_w gradually increased till reaching 2.5-3.0 g hydrocolloid/100g rice flour (RF) in the bread formula.

1. INTRODUCTION

Wheat (*Triticum aestivum* L) belongs to the family Gramineae (Poaceae) commonly known as the grass family. Where its grains are milled and converted to flour, which is used as a base material for baked products. Gluten is the main protein of wheat grains representing 85-90% of the total protein **(Wieser, 2007).** Where gluten is protein water-insoluble and consists basically of gliadin and glutenin during the kneading process forming the matrix of bread **(Rai *et al*., 2018).** The glutenin-gliadin ratio in wheat flour (WF) plays an important role in the rheological properties. Gliadin gives the dough viscous properties and allows it to stretch. At the same time glutenin improves dough cohesion and gives it strength and flexibility **(Wieser, 2007).**

Celiac disease (CD) is a chronic disease in which dietary gluten activates an immune response in the small intestine, leading to epithelial cell loss **(Atlasy *et al*., 2022).** This disease affects 0.5% to 5.6% of the population of the Mediterranean basin countries, especially Turkey, Tunisia, and Egypt, as 1% of the world's population suffered from this disease **(Machado, 2023).** Thus, gluten-free rice flour (RF) is widely used for such patients in the manufacture of baking products instead of wheat flour **(Roman *et al.,* 2019)**. However, gluten-free (GF) bread dough is often more liquid than wheat and, in most situations, is not moldable due to its viscosity.

Rice (*Oryza sativa* L.) flour is suitable for celiac patients because it has numerous unique qualities such as ease of digestion, white color, bland taste, and hypo-allergenicity **(Roman *et al*., 2019).** Nonetheless, rice bread (RB) has a higher staling rate (SR), higher crumb hardness (CH), and a lower specific volume (SV) than wheat bread (WB).

Plant mucilage derived from vegetable waste, such as taro (*Colocasia esculentus* L.), mallow (*Corchorus olitorius* L.), and okra (*Abelmoschus esculentus* L.), is widely used as a hydrocolloid in the production of GF products **(Shahzad *et al*., 2020; Elzoghby *et al*., 2023).** Because of its high mucilage content, okra was selected as one of the numerous mucilaginous vegetables. Okra mucilage (OM) is a random coil polysaccharide consisting of galactose, rhamnose, and galacturonic acid **(Alamri, 2014).** It can be used as emulsifier and thickening or adhesive material in GF flours to manufacture gluten-free baking products **(Gemede *et al*., 2018; *Liu et al.*, 2021).**

So, this work was designed to monitor the impact of the addition of mucilage extracted from okra industrial waste, as a novel natural ingredient, on the rheological characteristics and water activity of gluten-free rice bread (GFRB) compared with synthetic carboxymethyl cellulose (CMC).

2. MATERIALS AND METHODS

2.1. Materials

Broken rice (*Oryza sativa* L.) kernels were obtained from a private rice mill in Tanta, Al-Gharbia Governorate, Egypt. Wheat (*Triticum aestivum* L.) flour (72% extraction) was purchased from North Cairo Flour Mills Co, a Holding Company for Food Industries, in Egypt.

Okra (*Abelmoschus esculentus* L.) pods were kindly provided by the Horticulture Research Institute, Agricultural Research Center at Giza, Egypt. While Carboxymethyl cellulose (CMC) was obtained as a gift from Gluten-Free Center, Food Technology Research Institute, Agriculture Research Center at Giza, Egypt.

Instant active dry yeast (Lesaffre, S. L.L. Co., Marcq, France), egg white powder (Egypt Basic Industries Corporation), margarine (IFFCO Co., Suez, Egypt), table salt (NaCl), and sugar (Sucrose) were bought from the local market of Zifta City, Al-Gharbia Governorate, Egypt.

2.2. Methods

2.2.1. Preparation of rice flour and okra mucilage

Rice flour (RF) was prepared by the semi-dry grinding process **(Yeh 2004),** while okra mucilage (OM) was extracted by cooled water at a ratio of 1:2 (w/v) in a refrigerator at 5^{o} C for 24 hrs **(Machine *et al*., 2020).**

2.2.2. Preparation of the rice bread

Rice bread (RB) was prepared with different levels of CMC or OM as hydrocolloid agent (Table 1). The bread was prepared by mixing the hydrocolloid agent (CMC or OM) with the appropriate amount of water to form a suspension. The dry instant yeast was dissolved in about 50 ml water containing 10 g sugar and incubated at 35^{0}C for 3 min to activate the yeast. The dry ingredients such as the rice flour (RF), egg white powder, and salt were mixed together with margarine. Then the hydrocolloid suspension, the activated yeast, and the appropriate water were added and mixed together in a planetary mixer at low speed making dough. The dough was fractionated in portions of 60 g each, placed in the molds previously greased with margarine, and sprinkled with flour. The dough was subjected to fermentation in an incubator at 25±2°C, relative humidity 85% for 30 minutes. The fermented dough was baked in an electric oven at 180ºC for 30 minutes. Then, the loaves were cooled at room temperature (25 ± 2°C), removed from the molds and cooled for further analysis.

Table (1): Blends of rice flour fortified with different levels of hydrocolloid agents (CMC and OM)

Treatments	Ingredients (g/100g rice flour)*									
	WF	RF	CMC	OM	Water	Sugar	Salt	Yeast	Egg white	Margarine
WB (T_1)	100	-	-	-	75*	12	2	4	10	10
RB (T_2)	-	100	-	-	150	12	2	4	10	10
RB (T_3)	-	100	0.50	-	150	12	2	4	10	10
RB (T_4)	-	100	1.00	-	150	12	2	4	10	10
RB (T_5)	-	100	1.50	-	150	12	2	4	10	10
RB (T_6)	-	100	2.00	-	150	12	2	4	10	10
RB (T_7)	-	100	2.50	-	150	12	2	4	10	10
RB (T_8)	-	100	3.00	-	150	12	2	4	10	10
RB (T_9)	-	100	-	0.50	150	12	2	4	10	10
RB (T_{10})	-	100	-	1.00	150	12	2	4	10	10
RB (T_{11})	-	100	-	1.50	150	12	2	4	10	10
RB (T_{12})	-	100	-	2.00	150	12	2	4	10	10
RB (T_{13})	-	100	-	2.50	150	12	2	4	10	10
RB (T_{14})	-	100	-	3.00	150	12	2	4	10	10

*WF: Wheat flour; RF: rice flour; OM: okra mucilage; CMC: carboxymethyl cellulose

2.2.3. Texture profile analysis (TPA).

Texture Profile Analysis (TPA) was carried out on fresh and stored bread (after 24 and 48 hrs) in Bread and Pastries Laboratory, Food Technology Research Institute, Agricultural Research Center at Giza, Egypt, using the Brookfield CT3 instrument (Brookfield Engineering Laboratories, Inc., MA 02346-1031, USA) according to **AACC (2010).** The TPA curve was used to compute hardness (N), cohesiveness, gumminess (N), chewiness (mj), springiness (mm), and resilience as described in the operating instruction manual.

2.2.4. Determination of water activity (a_w) of the prepared bread

The water activity (a_w) of the freshly prepared loaf was determined at room temperature after 24, and 48 hrs of storage using a Decagon Aqualab Meter Series 3TE (Pullman, WA, USA) according to **Shahidi *et al.* (2008).**

2.2.5. Statistical analysis

The data represent the mean (M) and standard deviation (SD) of three successful experiments using SPSS (version 26 IBM SPSS Statistics Inc., Chicago. USA). The data was treated to a one-way analysis of variance (ANOVA) to find differences across samples using Tukey post hoc multiple comparison tests were used ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Texture profile analysis (TPA) of the bread fortified by OM or CMC

Texture analysis is normally used to determine food quality and acceptance **(Bourne, 2003).** It can be measured by subjective method via expert panelists or by the objective method using instrumental measurements **(Yang, 2016).** The results of Table (3) display the textural analysis profile (TPA) of rice bread (RB) containing different levels of synthetic CMC or natural OM as a hydrocolloid agent compared with wheat bread (WB). The data reveal that the hardness, chewiness, and gumminess of most bread samples are gradually increased by extending the storage period. The highest values for these factors (2.61, 10.90 and 3.41 for fresh WB and 18.44, 28.50 and 16.92 for fresh RB) appeared after 48 hrs of storage at room temperature. At the same time, the cohesion, resilience, and springiness of the bread decreased. The data also show that WB (T1) was superior in all measurements to RB (T2). However, the characteristics of RB improved by increasing the hydrocolloid agent percentage of the dough mixture, as the RB containing 3.0 OM/100g RF (T14) gave similar values to the WB (T1).

Hardness is defined as the force required for biting bread sample. Where, the hardness of the loaf bread is decreased with the incorporation of hydrocolloid agents (CMC or OM). The hardness of the rice bread (RB) is decreased with the incorporation of hydrocolloid agents either CMC or OM. For example, the hardness of RB (T2) decreased from 7.14 N to 2.58 N when the dough formula contained 3.0g OM/100g rice flour (T14) (Table 2 and Fig.1 -A).This might be related to the water colloidal CMC and OM having a high water retention capacity, which delays the crumb hardness (CH) and starch retrogradation **(Barcenas *et al*., 2004).** These results are in agreement with those of **Mohammadi *et al.* (2014)** on bread and **El-Sayed *et al.* (2014)** on the cake. Also, the hardness increases as storage period increased due to moisture loss and starch retrogradation **(Lazaridou *et al*., 2007).**

Cohesiveness is determined from the area of work during the second compression divided by the area of work during the first compression **(Bourne, 2003).** The cohesiveness is 0.76 for fresh RB (T2) which decreased due to extending the storage period reaching 0.61 after 48 hrs. On the other hand, it increases as the hydrocolloid agent increase in the rice dough formula. The maximum value (1.08 and 1.09) is recorded by T7 and T8 samples containing CMC at 2.5 and 3.0% respectively, followed by RB (T14) containing 3.0 g OM/100g RF (Fig. 1- B). The bread with low cohesiveness makes it more likely to crumble, and therefore less palatable to consumers **(Liu *et al*., 2018).**

The resilience (elasticity) shows the ability of the product to recover its original form. It increased gradually in RB samples with incorporated hydrocolloid (CMC or OM) in the dough formula (Fig. 1- C). By analogy, resilience decreased due to extending the storage period. These results are consistent with **Mohammadi *et al*. (2014).** As the bread formulations containing CMC gum have higher elasticity than that of control bread either in fresh or during storage. This is probably due to the physicochemical properties of hydrocolloids such as high water solubility, plasticity, elasticity, and viscosity **(Mohammadi *et al.*, 2014; Be Miller *et al*., 1993).**

Springiness is defined as the distance recovered by the sample in height during the time between the end of the first compression cycle and the beginning of the second one. Gumminess is a product of hardness and cohesiveness, while chewiness is a product of hardness, cohesiveness, and springiness. In general, consumers prefer bread that has low chewiness and gumminess **(Abd-Elkader *et al*., 2021).**

Table (2): Texture profile analysis (TPA) of wheat bread (WB) and rice bread (RB) with different levels of carboxymethyl cellulose (CMC) and okra mucilage (OM) as hydrocolloid agents at 0, 24, and 48 hrs of storage

Treatment*	Parameters																	
	Hardness (N)			Cohesiveness			Resilience			Springiness (mm)			Chewiness (mJ)			Gumminess (N)		
	Zero time	24 hrs	48 hrs	Zero time	24 hrs	48 hrs	Zero time	24 hrs	48 hrs	Zero time	24 hrs	48 hrs	Zero time	24 hrs	48 hrs	Zero time	24 hrs	48 hrs
WB T_1	1.97	2.27	2.61	1.03	0.96	0.85	0.71	0.54	0.50	3.71	3.51	3.44	7.50	10.80	10.90	2.50	3.24	3.41
RB T_2	7.14	10.66	18.44	0.76	0.66	0.61	0.45	0.39	0.37	1.99	1.48	1.33	15.10	28.50	50.90	7.12	9.03	16.92
RB T_3	5.51	7.60	8.85	0.86	0.78	0.72	0.48	0.43	0.41	3.35	3.26	3.03	10.90	16.20	19.20	4.03	4.54	5.23
RB T_4	5.16	6.18	7.48	0.98	0.84	0.76	0.57	0.51	0.40	3.39	3.30	3.21	14.50	15.90	17.10	3.92	4.45	4.96
RB T_5	4.26	5.01	5.97	0.99	0.87	0.77	0.66	0.58	0.49	3.49	3.40	3.28	13.60	14.80	16.50	3.11	3.37	4.74
RB T_6	2.58	3.01	3.37	1.01	0.92	0.82	0.70	0.61	0.52	3.68	3.50	3.25	8.50	11.20	15.40	2.65	4.36	4.70
RB T_7	7.38	8.99	10.03	1.08	1.03	0.97	0.75	0.69	0.67	3.01	2.88	2.34	10.43	13.36	17.38	4.23	3.98	4.11
RB T_8	9.22	11.67	14.11	1.09	1.07	1.07	0.79	0.67	0.63	2.96	2.51	1.41	15.23	17.69	18.41	4.96	4.63	4.12
RB T_9	6.45	9.69	12.42	0.82	0.72	0.56	0.49	0.44	0.40	3.29	2.85	2.64	21.60	27.20	46.40	6.30	8.03	13.81
RB T_{10}	6.3	8.57	9.4	0.85	0.75	0.64	0.55	0.47	0.42	3.43	3.17	2.80	11.90	15.50	29.30	3.39	4.43	8.74
RB T_{11}	5.21	6.15	7.32	0.91	0.85	0.66	0.67	0.47	0.43	3.49	3.22	2.90	10.30	14.70	26.90	3.28	4.33	7.18
RB T_{12}	5.46	6.32	6.33	0.99	0.89	0.71	0.70	0.49	0.45	3.49	3.38	3.08	9.20	13.90	19.50	2.96	4.23	6.33
RB T_{13}	3.87	4.37	4.47	0.99	0.93	0.73	0.78	0.50	0.46	3.65	3.47	3.35	6.70	12.40	16.50	2.34	3.76	4.73
RB T_{14}	3.48	3.83	3.92	1.01	0.96	0.87	0.74	0.65	0.48	3.74	3.49	3.36	6.00	7.10	15.20	2.08	2.68	4.63

*T_1= wheat bread (WB); T_2= rice bread (RB), T_3= RB containing 0.5% CMC, T_4= RB containing 1% CMC, T_5= RB containing 1.5% CMC, T_6= RB containing 2% CMC, T_7= RB containing 2.5% CMC, T_8= RB containing 3% CMC, T_9= RB containing 0.5% OM, T_{10}= RB containing 1% OM, T_{11}= RB containing 1.5% OM, T_{12}= RB containing 2% OM, T_{13}= RB containing 2.5% OM, T_{14}= RB containing 3% OM

The data of Table (2) and Fig (1- E & F) display that the best fresh bread is RB T14 (formula with 3.0g OM/100g RF) had the lowest chewiness and gumminess values (6.0 and 2.08), followed by T13 (formula with 2.5g OM/100g RF). These breads are better than standard bread WB (T1). The fresh RB contains 2.0g CMC/100g RF (T6) has chewiness and gumminess values close to the WB (T1). The RB that containing 2.0g CMC/100 g RF is enough good. However, the bread containing 3.0g OM/100g RF as improver hydrocolloid agent is better. These results are affirmed by **Lazaridou *et al.* (2007)** who stated that the use of 3.0% CMC leads to lower bread quality.

3.2. Water activity (a_w) of the bread fortified by OM or CMC

Water activity (a_w) affects the quality of the loaf including texture, taste, odor, volume, and flavor **(Ren *et al*., 2020),** which is an important factor in bread shelf life **(Hassan *et al*., 2020).** The higher the a_w of the bread, the rate the spoilage is faster. So, the a_w could be used as an indicator of the speed of spoilage. The a_w of fresh WB (T1) and RB (T2) is 0.898 and 0.885, respectively (Table 3 and Fig. 2). These values are in accordance with **Hager *et al*. (2012),** who stated that a_w of fresh bread ranges from 0.80 to 0.98. The a_w of fresh bread increases due to the increasing proportion of CMC or OM in the formula of the bread (Fig 2). Also, it rises after 24 and 48 hrs of storage at room temperature. There are no significant differences (p>0.05) in a_w between fresh normal WB (T1) and RB (T2). On the other hand, there are significant differences (p<0.05) in the a_w among the prepared bread samples containing gums compared to the control depending on the type and amounts of hydrocolloid agents. These results are in line with what was mentioned in the literature **(Jideani and Bello 2009; Yang, 2016).** This could be related to the absence of a gluten network in rice dough. It is clear that as the storage period extend the (a_w) of the bread gradually increased with no differences among them (p>0.05). This result agrees with those reported by **Lazaridou *et al*. (2007).**

4. CONCLUSION

In conclusion GF bread can be prepared by using CMC as an artificial colloidal agent or OM as a natural colloidal one at a concentration of 2.0g CMC / 100 g RF or 2.5g OM/100g RF. The quality of RB can also be improved by increasing the addition of OM up to 3.0g OM/100g RF. The a_w of the bread increases by adding the hydrocolloid agents.

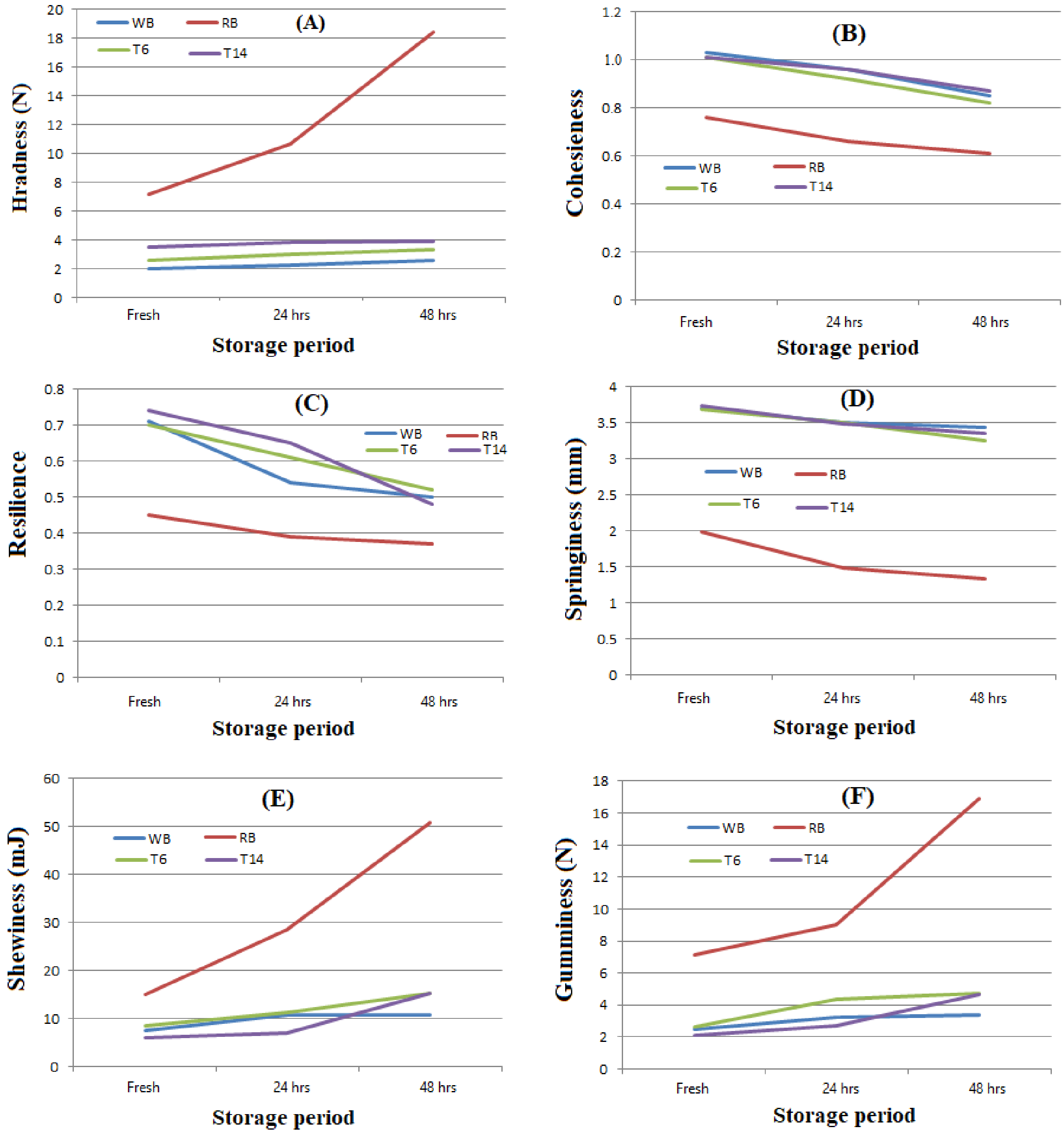

Fig. 1. Texture profile analysis (TPA) of wheat bread (WB) and rice bread (RB) containing 2.0 g CMC/100g RF (T6) or 3.0g OM/100g RF (T14) after 24, and 48 hrs of storage

Table (3): Water activity (a_w) of wheat bread (WB) and rice bread (RB) with enriched with carboxymethyl cellulose (CMC) or okra mucilage (OM) as hydrocolloid agents after 24, and 48 hrs of storage

Treatment*	Storage time		
	Fresh	24 hrs	48 hrs
WB T_1	0.898 ± 0.004^{cdef}	0.892 ± 0.003^{e}	0.884 ± 0.002^{e}
RB T_2	0.885 ± 0.006^{f}	0.874 ± 0.004^{f}	0.862 ± 0.002^{f}
RB T_3	0.890 ± 0.007^{def}	0.894 ± 0.003^{e}	0.909 ± 0.002^{cd}
RB T_4	0.897 ± 0.002^{cdef}	0.901 ± 0.005^{bcde}	0.910 ± 0.002^{bcd}
RB T_5	0.903 ± 0.006^{abcd}	0.905 ± 0.006^{abcde}	0.910 ± 0.07^{bcd}
RB T_6	0.907 ± 0.006^{abc}	0.912 ± 0.008^{abcd}	0.912 ± 0.005^{bcd}
RB T_7	0.913 ± 0.003^{ab}	0.916 ± 0.002^{ab}	0.919 ± 0.003^{abc}
RB T_8	0.915 ± 0.004^{a}	0.918 ± 0.003^{a}	0.923 ± 0.004^{a}
RB T_9	0.886 ± 0.006^{ef}	0.895 ± 0.005^{e}	0.902 ± 0.001^{d}
RB T_{10}	0.893 ± 0.004^{def}	0.897 ± 0.006^{de}	0.903 ± 0.004^{d}
RB T_{11}	0.900 ± 0.001^{bcde}	0.901 ± 0.006^{cde}	0.911 ± 0.002^{bcd}
RB T_{12}	0.904 ± 0.001^{abcd}	0.907 ± 0.002^{abcde}	0.916 ± 0.002^{abc}
RB T_{13}	0.908 ± 0.001^{abc}	0.914 ± 0.002^{abc}	0.919 ± 0.001^{ab}
RB T_{14}	0.915 ± 0.003^{a}	0.917 ± 0.004^{a}	0.923 ± 0.001^{a}

T_1= wheat bread (WB); T_2= rice bread (RB), T_3= RB containing 0.5% CMC, T_4= RB containing 1% CMC, T_5= RB containing 1.5% CMC, T_6= RB containing 2% CMC, T_7= RB containing 2.5% CMC, T_8= RB containing 3% CMC, T_9= RB containing 0.5% OM, T_{10}= RB containing 1% OM, T_{11}= RB containing 1.5% OM, T_{12}= RB containing 2% OM, T_{13}= RB containing 2.5% OM, T_{14}= RB containing 3% OM.
Values are Means (M) ± standard deviation (SD) of three successful trails
In a column, means having the same superscript letters are not significantly different at 0.05% level

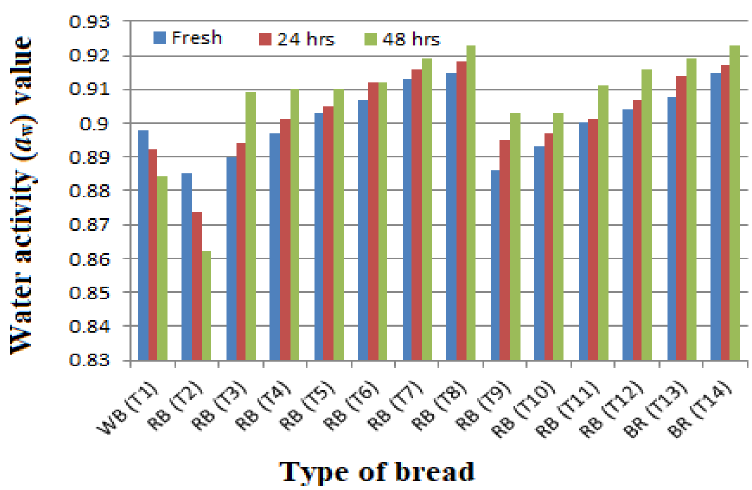

Fig. 2. Water activity (a_w) of wheat bread (WB) and rice bread (RB) enriched with carboxymethyl cellulose (CMC) or okra mucilage (OM) after 24 and 48 hrs of storage

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تأثير صمغ البامية (*Abelmoschus esculentus* L) على الخواص الريولوجية والنشاط المائي لخبز الأرز

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الملخص

تم اجراء هذا البحث لدراسة تأثير كربوكسي ميثيل السليلوز (CMC) كعامل غرواني مائي اصطناعي وصمغ البامية (OM) كعامل طبيعي على الخصائص الانسيابية لخبز الأرز (RB) مقارنة بخبز القمح (WB). كما تم تحديد النشاط المائي (a_w) لعينات الخبز المحضرة. لذلك ، تم استخدام جهاز CT3 Texture Analyzer لتحديد الخصائص الانسيابية (TPA) وتم استخدام جهاز Decagon Aqualab Meter Series 3TE لتحديد النشاط المائي (a_w). أظهرت النتائج أن التماسك والمرونة والربائية في خبز القمح الطازج (T1) أعلى من تلك الموجودة في خبز الأرز، والتي تقل عن طريق زيادة فترة التخزين. من ناحية أخرى ، فإن صلابة ومضغ وصمغ خبز القمح الطازج (T1) أقل من تلك الموجودة في خبز الأرز (T2). تنخفض قيمة هذه الخصائص بشكل عام في خبز الأرز الطازج عن طريق زيادة نسبة العوامل المائية الغروانية المضافة (CMC أو OM). تم الحصول على أفضل النتائج بإضافة 2 جرام كربوكسي ميثيل سليلوز لكل 100 جرام دقيق أرز أو 3 جرام من صمغ البامية لكل 100 جرام دقيق أرز في خلطة العجين. يبلغ نشاط الماء في خبز القمح الطازج 0.898 (T1) و الذي انخفض تدريجياً ليصل إلى 0.884 بدون فرق معنوي. لوحظ نفس الاتجاه أيضًا بالنسبة لخبز الأرز (T2). على العكس من ذلك ، زاد النشاط المائي (a_w) تدريجيا بزيادة العامل الغرواني المائي في تركيبة خبز الأرز حتى وصل إلى 2.5-3.0 جم غرواني مائي / 100 جم دقيق أرز في خلطة الخبز.

مجلة العلوم الزراعية والبيئية المستدامة