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EFFECT OF TEMPERING TIME, WATER TEMPERATURE AND ADDED ENZYMES ON FLOUR YIELD AND CHARACTERISTICS OF AUSTRALIAN WHEAT GRADE

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ABSTRACT: This recent study was to relate tempering time, tempering water temperature and added enzymes to flour yield, chemical analysis, rheological characters and pan-bread quality. Results showed that, the highest significant grain protein percentage was obtained with 20℃ tempering temperature and 16 hours tempering time (15.27%). Whereas, the least significant grain protein was recorded with any of 20℃ tempering temperature and 24 hours tempering time (14.90%) or 40℃ temperature and 16 hours tempering time (14.97%). Protein percentage was highest with 16 hours tempering and no addition of enzyme (15.23%). While, the least significant protein values were presented with 24 hours tempering and the addition of any of (E_1) or (E_2) enzymes (14.93%). The highest significant value of falling number was expressed when tempering temperature was 20°C with adding hemicellulase + xylanase (E_1) enzymes (587.8 sec.). The highest significant gluten (%) value was obtained when tempering temperature was 20℃ without any addition of enzymes (34.30%). The highest significant extraction (percentage) value obtained when tempering temperature was 40℃ with addition of the enzymes; hemicellulase + xylanase + fungal α-amylase (71.37%). Whereas, the least significant values of that variables were obtained with 20℃tempering temperature with the addition of hemicellulase + xylanase enzyme (66.43 %). Significantly, the highest value of extraction percentage was resulted from 24 hours tempering and addition of enzyme Hemicellulase + Xylanase + fungal α- amylase (71.60 %). The highest significant values of stability time was presented with the combination of 20℃ with eight hours of tempering and the addition of (E1) enzymes (36:25 mm:ss). While, the least value of stability time was that expressed by the combination of 20℃ with eight hours tempering and the addition of (E_2) enzymes (8:36 mm:ss). The highest significant values of Farinograph quality number were presented with the combination of 20℃ with 8 hours of tempering with the addition of (E_1) enzymes (430 mm). The highest significant values of loaf weight were obtained when tempering temperature was 40℃ with 16 hours tempering and no addition of enzymes (258.0 g). Significantly similar values of loaf volume (cm^3) and specific volume (cm^3/g) was presented with 40℃ tempering temperature, 16 or 24 hours of tempering and adding Hemicellulase + Xylanase enzymes or Hemicellulase + Xylanase + fungal α- amylase enzymes (1605.0 or 1580.0 cm^3 and 6.472 or 6.575 cm^3/g, respectively). The highest significant values of peak force were presented with the combination of 20℃ or 40℃ with eight hours of tempering time for five days storage (922.0 g). The highest significant values of peak force were presented with the combination of eight hours of tempering time and five-days of storage time with no addition of enzymes (1125.0 g). Enzymes added to tempering water significantly improved the overall acceptability as 6.311, 7.067 and 7.578, for E_0, E_1 and E_2, respectively. Best-fit equations that related grain properties of Australian wheat grade to tempering time, tempering water temperature and added enzyme and the out-come might be summarized as follow:

Stability R^2 =0.77

Y= -919.659 + 11.383[Test weight (kg/h)] + 5.095[Particle size index (psi %)] - 1.628[Tempering time] + 0.243[Tempering temperature] - 8.881[Tempering added enzyme].

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Loaf volume $R^2 = 0.78$

Y= 17956.161 - 212.978[Test weight (kg/h)] - 12.863[Particle size index (psi %)] + 18.246[Tempering time] +1.430 [Tempering temperature] + 102.621[Tempering added enzyme].

Key words: Tempering time, water-tempering temperature, added enzymes, and flour yield.

INTRODUCATION

Wheat is the most important grain crop worldwide based on acreage and total production (Shahbandeh, 2021). The global wheat produced reaches 772 million metric tons, with an increase of about ten million tons, compared to previous marketing year (Shahbandeh, 2021). Approximately 70% of worldwide produced wheat is used for food (Dendy and Dobraszczyk 2001). Milling is very important steps in wheat processing. Wheat milled to flour, that then made to variable products such as bread, cakes, cereals, macaroni, and noodles. Hard and soft wheat flours of high protein content (>11%) are preferred in wet milling to co-produce vital gluten and starch. Whereas, Wheat starch is used to produce modified starch (Abdulvahit 2004).

Wheat grain quality depends upon, cultivar, climatic conditions, year and process of harvest and storage conditions. Wheat grain quality is expressed by a variety of physical and chemical traits such as test weight, kernel weight, particle size index (PSI), moisture content, ash content and protein content. The major determinants of wheat quality are endosperm texture and protein content. Endosperm texture has a profound effect on milling, baking and end-use quality. As a varietal character, endosperm hardness is also influenced by environment. Grain hardness is mainly influence by various physical and chemical factors like protein, virtuousness, kernel size, water-soluble pentosans, moisture content and lipids (Pasha *et al*, 2010).

Flour yield and flour properties, among other things, strongly related to wheat kernel properties, especially to mechanical properties. Those include kernel color, virtuousness, mass, shape, test weight, density, size and size uniformity. These properties depend on many related factors, such as genetic make-up, agro-technical methods or agro environmental conditions. Based on such properties, we can recommend the end -use of wheat (Dziki and Laskowski. 2005).

Tempering is the process of adding water to wheat before milling to toughen the bran, mellow the endosperm of the kernel, and thus improve the efficiency of flour extraction. Numerous researchers have reported studies on water uptake, movement, and diffusion during tempering (Stenvert and Kingswood 1976, 1977; Song *et.al* 1998; Kang and Delwiche, 2000; Delwiche 2000). Tempering temperature, moisture, and duration affect the rate of moisture uptake to kernels. Also, wheat cultivar, initial kernel water content, and kernel size influence that rate (Posner and Hibbs 1997). Although the rate of water penetration into wheat kernel during tempering varied for different cultivars, the mode of movement was essentially the same (Stenvert and Kingswood 1976). The rate of moisture uptake is also, influenced by endosperm structure and protein content along with distribution (Stenvert and Kingswood 1977).

In the process of wheat milling, traditionally conditioning was applied before grinding to enhance the separation of the germ and the external layers from the starchy and proteinous endosperm, that constitute the refined flour. Conditioning (also referred to as tempering) is known as the process of adding small amount of water to wheat kernels thus improves the efficiency of flour extraction. This step is also fundamental for the technological quality of the flour, since, it represents complex system of chemical-physical-enzymatic reactions. Changes occur, partly not yet completely known (Kweon *et al*, 2009).

Enzymatic tempering is a modified tempering process that involves cell wall degrading enzymes which are mixed with the tempering water at room temperature. This in turn, toughens the bran layer and mellows the endosperm layer, facilitating better scratching of endosperm from the bran layer. Additionally, the enzymes also hydrolyze complex components present in the bran layer, like arabinoxylans to release free sugars and allow it available to enhance its baking quality. Water uptake during the tempering process occurs in two distinct phases: adherence to the surface of the kernel and diffusion into the kernel (Lamsal and Faubion 2009).

The aims of the present study were to relate wheat commercial grade, tempering time, tempering water temperatures, and added tempering enzymes to flour yield and flour quality characteristics.

MATERIALS AND METHODS

The main objectives of this recent study were to relate wheat commercial grade, tempering time, tempering water temperature and added enzymes to flour yield, chemical analysis, rheological characters and pan-bread quality. Facilities of cereals quality lab., Crop Science Department, Alexandria University and labs of quality control of the Arabian Milling and Food Industries (Abo - Donkol) company were used to carry- out the recent work.

The studied treatments included three main factors. These were; a) commercial wheat grade, represented by Australian, Russian and Romanian grades, b) tempering water temperature represented by two levels; 20 and 40℃, c) tempering time represented by three levels; 8,16 and 24 hours, and d) added enzymes to tempering water, represented by three levels; (E_0) no enzymes, (E_1) hemicellulase + xylanase and (E_2) hemicellulase + xylanase + fungal α-amylease. These factors represented 18 treatments as compilations among the studied factors (Table 1).

Each treatment was applied to a sample of wheat weighted 50 kg. Enzymes were of commercial form prepared by Novozymes Company. Tempering of samples was accomplished in rotart incubator. A Laboratory mill (C D1 auto Chopin) finished milling according to the methods described in AACCI Approved Method 26-70.01 of the AACC (2000).

Table 1: Compilations among tempering water temperature, tempering time and added enzyme.

Code	Tempering water temperature	Tempering time	Added enzyme
T1	20℃	8hours	E_0
T2	20℃	8hours	E_1
T3	20℃	8hours	E_2
T4	20℃	16hours	E_0
T5	20℃	16hours	E_1
T6	20℃	16hours	E_2
T7	20℃	24hours	E_0
T8	20℃	24hours	E_1
T9	20℃	24hours	E_2
T10	40℃	8hours	E_0
T11	40℃	8hours	E_1
T12	40℃	8hours	E_2
T13	40℃	16hours	E_0
T14	40℃	16hours	E_1
T15	40℃	16hours	E_2
T16	40℃	24hours	E_0
T17	40℃	24hours	E_1
T18	40℃	24hours	E_2

E_0: Control, E_1: Hemicellulase + xylanase, E_2: Hemicellulase +xylanase+fungal α- amylase.

1. Physical analysis

1.1 Test weight of wheat was determined according to AACCI2000 (method 55-10.01) after dockage is removed.

1.2 Specific volume of wheat was determined according to AACCI2000 (method 55-50.01).

1.3 Impurities, shrunken and broken kernels, foreign material, insects test, total defects and cleaning test were determined according to AACCI2000 (method 28-01-01).

1.4 Particle size index for wheat hardness according to AACCI 2000 (method 55-30, 01).

2. Chemical analysis

2.1; Moisture percentage; (According to AACC-44-01.01, 1999).

2.2; Crude protein percentage; (According to AACC.46-11.02, 1999).

2.3; Ash percentage; (According to AACC.08-21.01, 2000).

3. Physio- chemical properties

3.1; Gluten content (%); (According to AACC 38-12.02, 2000).

3.2; Falling Number (sec): (AACC 56-81.03, 1999).

4. Rheology characteristics

4.1; Farinograph; (According to AACC54-21.01, 2000).

4.2; Alveograph; (According to AACC54-30.02, 2000).

5- Extraction rate

The extraction rate of flour calculated automatically by milling scales as follows:

Extraction rate = Weight of flour (kg) / weight of tempered wheat (kg) ×100

6- Bread making

6.1 Pan-Bread

Pan-bread was prepared by mixing one kilogram of flour, 20gm active dry yeast, 80gm sugar and 5 gm sodium chloride, placed in the mixer and mixed with water until optimum consistency was obtained. The dough was removed from the mixer and rounded, mounded and put in a pan (6 * 8 * 21cm) (lightly greased pan to prevent the sticking for resulted bread). Fermentation process was carried out for 60 min through three consecutive stages at 45 ℃, 90 relative humidity. After proofing, the pans were placed in oven at 200 ℃ for 30 minutes.

6-2 Bread Loaf Specific Volume

Each bread loaf was weighed in grams using analytical balance. Bread loaf volume was measured according to the rapeseed displacement method of measuring volume in cubic centimeters (cm^3) as described by AACC approved method 10-05-01 (2011). Specific volume calculated as cm^3/g by dividing the volume of the bread loaf by its weight.

6-3 Organoleptic evaluation of pan-bread

A panel of six judges evaluated the organoleptic characteristics of prepared breads. They assessed crust color, crumb color, flavor, texture, taste and overall acceptability, using a nine- point hedonic rating scale ranging from like extremely (9) to dislike extremely (1) for each characteristics as reported by Harinder *et al*, (1999). Key to bread score was liked extremely 9, liked moderately 7, neither like nor disliked 5, disliked moderately 3 and disliked extremely 1. The overall acceptability score determined by summing the score for each characteristic and computing the average.

6-4 Crumb firmness

The crumb firmness or hardness was determined using texture analyzer perten instruments TVT type 6700 (Huddinge, Sweden). Texture profile analysis was carried-out in a single compression cycle using 25mm diameter stainless steel cylinder probe. Bread firmness and staling rate recorded by measure the hardness or force. Measurements of TVT (peak force) were taken in the first, second, third, fourth and fifth day (According to AACCI (method 10-05, 01)). Physical characters of the Australian wheat grade were illustrated in Table 2.

Table 2: Physical characters of the studied wheat grade.

Wheat grade	**Australian wheat**
Test weight (kg/h)	79.50±0.500
1000 kernel weight (g)	38.10±0.400
Single kernel	
Weight (mg)	38.00±0.100
Diameter (mm)	3.100±0.500
Length (mm)	6.100±0.500
Particle size index (psi)%	15.00±1.000 Hard
Dockage (%)	0.900±0.200
Damaged kernel (%)	0.500±0.200
Foreign material (%)	0.800±0.200
Shrunken & Broken (%)	0.800±0.500
Total defects (%)	3.000±1.000
Vitreous Kernels (%)	85.00±5.000
Moisture	11.00±0.300
Crud protein	17.60±0.300
Gluten content	37.70±0.600
Gluten index	73.00±7.000
Falling Number (sec)	510.0±20.00

±: stand for standard error.

Statistical analysis

Numerical data were transformed to fit normal distribution before analysis. Mstat-c program version (2.10), was used for data analysis. Data were statistically analyzed using the analysis of variance technique as a CRD design. Means were compared using least significant difference test at 0.05 probability level (L.S.D 0.05) when F value was significant, According to Gomez and Gomez (1984). Regression equations were statistically estimated by Co Stat software (version 6.311).

RESULTS AND DISCUSSION

1. Chemical analysis

Chemical analysis of Australian wheat grains included the determination of moisture (%), crude protein (%) and ash (%). Protein percentage was highest with 16 hours tempering and no addition of enzyme (15.23%). While, the least significant protein values were presented with 24 hours tempering and the addition of any of (E_1) or (E_2) enzymes (14.93%). These values were not significantly different from the combination of eight hours tempering with any of no added enzymes (E_0) or adding hemicellulase + xylanase (E_1) (Table 3).

Irrespective of tempering time, water uptake into soft grain starchy endosperm is faster than into hard grain types. This was caused by enhanced capillary forces due to lower adhesion between the starch granules and the protein matrix of soft grain starchy endosperms (Mankey *et al*, 2011). Tyl, *et al*, 2019, noted that neither tempering time (four, eight, or 24 hours), nor tempering water tempering (30 or 45 ℃) had shown a pronounced effect on most flour properties indicating that water up-take was fast and not dependent on temperature. In general, tempering resulted in flour with less bran contamination but with minor losses in protein. Abo-Dief *et al*, 2019, concluded that, high quality flour and consequently highly acceptable pan-bread might be reached when Australian and Russian wheat grades were tempered for 12 and 24 hours, respectively. Kurt and Dizlek, 2022, reached that tempering reduced ash and protein contents of flour. Enzyme addition to tempering water might be accomplished by scarifying kernels before enzyme application. The latter process might help effective transport of enzyme to the aleuronic-endosperm interface. Moderately positive results in term of straight-flour yield with similar protein and flour color were obtained from enzyme tempering (Lamsal *et al*, 2008). The addition of combined enzymes cellulase, xylanase and pectinase to tempering water gave flour of higher protein content.

Table 3: Means of protein (%) for Australian wheat grade as affected by the first order interaction between tempering time and added enzyme.

Tempering time	**Protein (%)**		
	E_0	**E_1**	**E_2**
8h	14.95	14.92	15.18
16h	15.23	15.03	15.08
24h	15.08	14.93	14.93
L.S.D.$_{0.05}$	0.14		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

2. Physio-chemical properties

Response of gluten and falling number to tempering temperature × added enzymes interaction was presented in Table 4. The highest significant gluten (%) value was obtained when tempering temperature was 20℃ without any addition of enzymes (34.30%), Whereas, high tempering temperature of 40℃ resulted significantly in lower falling number values irrespective of the added enzymes. Meanwhile, the highest significant value of falling number was expressed when tempering temperature was 20℃ with adding hemicellulase + xylanase (E_1) enzymes (587.8 sec.).

Table 4: Means of wet gluten (%) and falling number (sec) of Australian wheat grade as affected by the first interaction between tempering water temperatures and added enzyme.

Tempering water temperature	**Added enzyme**	**Wet gluten (%)**	**Falling number (sec)**
20℃	E_0	34.30	577.9
	E_1	33.80	587.8
	E_2	34.16	576.4
40℃	E_0	33.97	521.4
	E_1	34.09	530.3
	E_2	33.90	512.8
L.S.D.$_{0.05}$		0.13	6.91

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Falling number (sec) was significantly affected by the interaction among tempering temperature × tempering time × added enzymes (Table 5). The highest significant values of falling number (sec) were obtained when tempering temperature was 20℃ with any of 16 or 24 hours tempering and no addition of enzymes (603.0 and 598.3 sec., respectively). Significantly similar values of falling number was presented with 20℃ tempering temperature, 24 hours of tempering and adding hemicellulase + xylanase (E_1) enzymes (589.7 sec.).

Table 5: Means of falling number (sec) of Australian wheat grade as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering water temperature	Tempering time	Falling number (sec)		
		E_0	**E_1**	**E_2**
20℃	**8h**	532.3	586.3	595.0
	16h	603.0	587.3	561.3
	24h	598.3	589.7	573.0
40℃	**8h**	551.7	534.3	479.3
	16h	554.0	516.3	490.7
	24h	458.7	540.3	568.3
L.S.D.0.05		11.96		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Increasing tempering time, affected flour yield and improved flour quality. Tempering to 15% moisture, provided better separation of mellowed endosperm from toughened bran and finally increased gluten strength. Commonly an improvement in flour quality was obtained with less ash content (Kweon, *et al, 2009*). Yamaguchi, *et al,* 2021, used tempering times of 13, 15, 17, 19, and 21 hours to trace the influence of tempering time on color, ash, gluten, and falling number. None of the used tempering times had influenced gluten strength. They reached that 17 hours tempering was the best tempering duration, beside, 13 hours for economic milling companies. Kurt and Dizlek, 2022, reached that tempering reduced ash and protein contents of flour, but gave an increase in gluten quality.

3. Extraction rate

Flour extraction rate is a measure of the percentage of the grain that is made into flour during the milling process. The extraction rate of flour calculated scales as follows:

Extraction rate =
Weight of flour ⁄ weight of tempered wheat ×100.

Table (6), illustrated the response of extraction rate to tempering time × added enzymes interaction. Significantly, the highest value of extraction percentage was resulted from 24 hours tempering and addition of enzyme Hemicellulase + Xylanase + fungal α- amylase (71.60 %), While the least extraction rate was resulted with eight hours tempering and the addition of Hemicellulase + Xylanase (66.00 %).

Table 6: Means of extraction rate (%) for Australian wheat grade as affected by the first order interaction between tempering time and added enzyme.

Tempering time	Extraction rate (%)		
	E_0	E_1	E_2
8h	69.85	66.00	69.90
16h	69.10	68.50	66.95
24h	68.65	68.60	71.60
L.S.D.$_{0.05}$	0.032		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Means of Extraction rate as affected by the second order interaction among tempering temperature × tempering time × added enzymes were illustrated in Table (7). The highest significant values of extraction were obtained when with tempering temperature was 40°C with 24 hours tempering time and the addition of Hemicellulase + Xylanase + fungal α- amylase enzymes (73.00 %). While the least value of extraction rate, was that expressed by the combination of 20°C with 16 hours tempering and the addition of Hemicellulase + Xylanase + fungal α- amylase enzymes (61.50%).

Table 7: Means of extraction rate (percentage) for Australian wheat grade as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering water temperature	Tempering time	Extraction rate (%)		
		E_0	E_1	E_2
20°C	**8h**	69.90	63.70	69.20
	16h	69.20	66.80	61.50
	24h	68.20	68.80	70.20
40°C	**8h**	69.80	70.20	70.60
	16h	69.00	70.20	70.50
	24h	69.10	68.40	73.00
L.S.D.$_{0.05}$		0.045		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Tempering toughens pericarp, and fewer small pericarp particles are formed during milling. However, as tempering moisture within the kernel increased, flour extraction rate decreased. Therefore, the tempering procedure should balance the level of flour extraction and acceptable level of bran in flour (Hook *et al*, 1982, a, b and c). Gaines *et al*, (1998), showed that, rain causes wheat kernels to swell. However, subsequent drying does not return some layers of the pericarp to their original pre-rain size, leaving some of the pericarp layers to exhibit a loose or puffed appearance. These changes cause decrease of grain density and test weight, but do not influence the flour yield. Rakszegi, *et al*, 2000, grains of harder endosperm produce higher flour extraction rate considering grain physical characters and improving details of milling steps, has recently improved flour yield and flour quality. Cellulase required 40°C and pH equal six.

Pectinase required 45℃ and pH equal five. Xylanase required 55℃ and pH equal five. The optimum tempering with enzymes was reached at a concentration of 143 EU for 12.4 hours at 28℃. An improvement in flour yield with enzymatic tempering reached about 4%. (Jha *et al*, 2020). (Lai *et al*, 2023). The addition of combined enzymes cellulase, xylanase and pectinase to tempering water gave flour of higher protein content, no improvement in flour yield (Yoo *et al*, 2009). Enzymes addition to tempering water (arabinase, celluase, β-glucanase, hemicellulase, xylanase and a fungal α-amylase) independently of working temperature increased the percentage of fine particles and showed that, enzymatic preparation gave a further increase in fine fraction leading to higher milling yield. Enzymatic conditioning was used to improve extraction rate and reduced fiber content of flour (Attia and obaid, 2023). Cellulase was added at rates of 24, 60, 0r 96 units/100gm of wheat. Overall, enzymatic conditioning levels, an increase in extraction rate was achieved relative to control (74.6 vs. 70.0% for the former and the latter, respectively).

4. Rheology characteristics

4.1. Farinograph parameters

The farinograph measurements, included, development time (DDT), water absorption (W), stability (S), degree of softening (10min after beginning) (Ds), degree of softening (ICC / 12 min after max.) (Ds (ICC)) and farinograph quality number (FQN). Means of Farinograph parameters as affected by the second order interaction among tempering temperature, tempering time and added enzyme were illustrated in Table (8). The highest significant values of development time were presented with the combination of 20℃ with 16 hours of tempering and no addition of enzyme (27:44 mm:ss). While, the least value of development time was that expressed by the combination of 20℃ with eight hours tempering and addition of (E_2) enzymes (5:25 mm:ss). The highest significant values of stability time was presented with the combination of 20℃ with eight hours of tempering and the addition of (E1) enzymes (36:25 mm:ss). While, the least value of stabilty time was that expressed by the combination of 20℃ with eight hours tempering and the addition of (E_2) enzymes (8:36 mm:ss). While, the highest significant value of degree of softening 10 min after beginning was obtained when tempering temperature was 20℃ without addition of enzymes and 16 hours tempering (37.00 FE). Whereas the highest significant value of degree of softening 12 min after max. were obtained when tempering temperature was 20℃ with the addition of hemicellulase + xylanase + fungal α- amylase enzymes and 24 hours tempering (45.00 FE). Meanwhile, the highest significant values of Farinograph quality number were presented with the combination of 20℃ with 8 hours of tempering with the addition of (E_1) enzymes (430 mm). Also, the least significant value of Farinograph quality number was that expressed by the combination of 20℃ with eight hours tempering and the addition of (E_2) enzymes (95mm).

Dough development time used as an indicator of mixing requirements of the farinograph at which, dough reaches maximum consistency. In cereal technology, wheats that have long dough-development time are considered strong (Bushuk *et al.*, 1969). The variation in dough rheology and bread making performance among wheat cultivars were largely determined by differences in protein quantity and composition (MacRitchie, 1992). Dough development time and stability value are indicators of the flour strength, with higher values suggesting stronger doughs (Wang *et al*., 2002). Some common predictor of dough strength include gluten index and empirical rheological tests such as farinograph and alveograph provide some information on dough extensibility as well as on overall strength (Carson and Edwards, 2009). Addition of xylanase enzyme to tempering water gave a positive impact on dough rheology characters and bread sensory evaluation attributes (Ahmad, *et al, 2013*).

Table 8: Means of Farinograph parameters for Australian wheat grade as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering temperature	Tempering time	DDT (mm:ss)			S (mm:ss)			Ds (FE)			Ds(ICC) (FE)			FQN (mm)		
		E_0	E_1	E_2	E_0	E_1	E_2	E_0	E_1	E_2	E_0	E_1	E_2	E_0	E_1	E_2
20°C	**8h**	9:24	9:39	5:25	22:57	36:25	8:36	7.00	4.00	30.00	30.00	45.00	62.00	177.0	425.0	95.00
	16h	27:44	18:11	9:15	33:10	33:17	12:20	37.00	13.00	13.00	17.00	13.00	41.00	430.0	374.0	133.0
	24h	6:17	7:11	7:59	30:41	12:30	11:56	10.00	8.00	16.00	27.00	42.00	45.00	177.0	151.0	136.0
40°C	**8h**	9:13	7:42	9:17	34:14	34:57	20:49	3.00	3.00	3.00	23.00	11.00	28.00	399.0	393.0	205.0
	16h	14:54	11:13	6:10	32:02	30:53	13:31	21.00	6.00	16.00	16.00	17.00	37.00	383.0	364.0	134.0
	24h	8:29	5:59	7:41	14:02	12:47	14:15	9.00	23.00	2.00	32.00	44.00	38.00	177.0	115.0	165.0
L.S.D.$_{0.05}$		3.717			8.161			5.221			11.37			91.14		

E_0: Control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase, DDT: Development time, S: Stability, DS: Degree of softening (10min after beginning), DS (ICC): Degree of softening (ICC / 12 min after max.), FQN: Farinograph quality number.

4.2. Alveograph parameters

Results of alveograph measurements, included maximum over pressure (P), average abscissa at rupture (L), deformation energy of dough (W), curve configuration ratio (P/L) and elasticity index (Ie). Means of dough deformation energy (10^{-4}J) as affected by the second order interaction among tempering temperature, tempering time and added enzyme were illustrated in Table (9). The highest significant values of dough deformation energy were presented with the combination of 20℃ with 24 hours of tempering and the addition of E_1 (Hemicellulase + Xylanase) enzyme (484.0 10^{-4}J), while, the least value of dough deformation energy was that expressed by the combination of 40℃ with 16 hours tempering and no addition of enzyme (E_0) (239.0 10^{-4}J).

Table 9: Means of Alveograph parameters (W) of Australian wheat grade as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering water temperature	Tempering time	W (10^{-4}J)		
		E_0	E_1	E_2
20℃	8h	465.0	403.0	413.0
	16h	475.0	464.0	332.0
	24h	344.0	484.0	402.0
40℃	8h	422.0	424.0	405.0
	16h	239.0	328.0	384.0
	24h	446.0	415.0	470.0
$L.S.D._{0.05}$		136.2		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase, W: deformation energy dough.

Yamaguchi, *et al*, 2021, used tempering times of 13, 15, 17, 19, and 21 hours to trace the influence of tempering time alveograph parameters. Non of the used tempering times had influenced extensibility ratio. They reached that 17 hours tempering was the best tempering duration, beside, 13 hours for economic milling companies. Kurt and Dizlek, 2022, reached that tempering gave an increase in gluten quality.

5. Physical properties of pan-bread

Physical properties of wheat grains included the determination of loaf weight (g), loaf volume (cm^3), specific volume (cm^3/g). Loaf weight (g), loaf volume (cm^3) and specific volume (cm^3/g) were significantly affected by the interaction among tempering temperature × tempering time × added enzyme (Table 10). The highest significant values of loaf weight were obtained when tempering temperature was 40℃ with of 16 hours tempering and no addition of enzymes (258.0 g). Significantly similar values of loaf volume (cm^3) and specific volume (cm^3/g) was presented with 40℃ tempering temperature, 16 or 24 hours of tempering and adding hemicellulase + xylanase enzymes or hemicellulase + xylanase + fungal α-amylase enzymes (1605.0 or 1580.0 cm^3 and 6.472 or 6.575 cm^3/g, respectively).

Table 10: Means of physical properties of pan-bread made from Australian wheat as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering temperature	Tempering time	Loaf weight (g)			Loaf volume (cm^3)			Specific Volume (cm^3 \g)		
		E_0	E_1	E_2	E_0	E_1	E_2	E_0	E_1	E_2
20℃	8h	249.2	242.5	247.2	1205.0	1452.5	1455.0	4.835	5.987	5.882
	16h	254.2	254.2	251.2	1320.0	1240.0	1435.0	5.195	4.878	5.713
	24h	246.5	243.0	246.5	1310.0	1425.0	1457.5	5.313	5.865	5.912
40℃	8h	232.2	231.5	229.2	1190.0	1252.5	1372.5	5.125	5.408	5.988
	16h	258.0	252.2	248.0	1320.0	1495.0	1605.0	5.117	5.925	6.472
	24h	245.25	242.0	240.2	1380.0	1495.0	1580.0	5.625	6.177	6.575
L.S.D.0.05		3.377			61.56			0.265		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Considering grain physical characters and improving details of milling steps, has recently improved flour yield and flour quality (Lai *et al*, 2023). Increasing tempering time, affected flour yield and improved flour quality. Tempering to 15% moisture, provided better separation of mellowed endosperm from toughened bran and finally increased gluten strength. Commonly an improvement in flour quality was obtained with less ash content (Kweon, *et al*, 2009). Abo-Dief, *et al*, 2019, concluded that, high quality flour and consequently highly acceptable pan-bread might be reached when Australian and Russian wheat grades were tempered for 12 and 24 hours, respectively. Kurt and Dizlek, 2022, reached that tempering gave an increase in gluten quality. Treating wheat kernels during tempering by enzymes, such as; cellulase, xylanase, and β-glucanase, had a positive effect on improving final products quality. Bread quality characters represented by volume, crumb and firming are major affected quality characters. Although of that, enzymes addition to tempering water has been less studied. The major objectives of enzymes use are improving milling and baking performance (Haros, *et al*, 2002, and Yoo, *et al*, 2009). Hille and schooneveld-Bergmaus, 2004, stated that, the addition of cellulase and cellobiohydrolyase to fungal and bacterial endoxylauase enzymes improved the tempering process. Cellulases open and break down cellulase, which inter twined with the arabinoxylan polymer, resulted in helping the endoxylanase activity. Such synergistic effect had a positive influence on bread volume and crumb softness. The addition of combined enzymes cellulase, xylanase and pectinase to tempering water gave flour of higher protein content, no improvement in flour yield, no improvement in loaf volume, and an improvement in bread firmness (Yoo *et al*, 2009).

6. Freshness of pan-bread

The ability of the bread to be stored with no stalling was measured as one of the most important factors for indicating the keeping quality of the resulted product. Pan-bread was stored for one, three and five days at room temperature. Means of pan-bread freshness indicated by power required for shearing (TVT) as affected by the second order interaction among tempering temperature, tempering time and the addition of enzyme were illustrated in Table (11). The highest significant values of peak force were presented with the combination of 40℃ with eight hours of tempering and no addition of enzymes (676.0 g), while, the least value of peak force was presented by the combination of 40℃, 16 hours tempering and the addition of E_2 (Hemicellulase + Xylanase + fungal α- amylase) enzyme (392.8 g).

Table 11: Means of freshness (TVT) of pan-bread made from Australian wheat as affected by the second order interaction among tempering water temperature, tempering time and added enzyme.

Tempering temperature	Tempering time	Peak force (g)		
		E_0	E_1	E_2
20℃	8h	672.9	545.3	447.1
	16h	614.0	611.9	424.8
	24h	608.5	490.5	402.3
40℃	8h	676.0	571.7	472.1
	16h	607.7	528.0	392.8
	24h	609.4	487.2	403.0
L.S.D.0.05		28.96		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Peak force values as affected by the interaction among tempering temperature × tempering time × storage days were presented in Table (12). The highest significant values of peak force were presented with the combination of 20℃ or 40℃ with eight hours of tempering time for five days storage (922.0 g). While, the least significant value of peak force was accompanied with the combination of 40℃ and 16 hours tempering and one-day storage (98.33 g).

Table 12: Means of freshness (TVT) of pan-bread made from Australian wheat as affected by the second order interaction among storage time, tempering water temperature and tempering time.

Tempering temperature	Tempering time	Peak force (g)		
		1day	3days	5days
20℃	8h	143.7	599.7	922.0
	16h	150.7	615.5	884.6
	24h	146.2	472.7	882.3
40℃	8h	143.7	654.2	922.0
	16h	98.3	550.0	880.2
	24h	141.5	475.8	882.3
L.S.D.0.05		28.96		

Means of bread freshness as affected by the second order interaction among tempering time, storage time and added enzyme were illustrated in Table (13). The highest significant values of peak force were presented with the combination of eight hours of tempering time and five-days of storage time with no addition of enzymes (1125.0 g). While, the least value of peak force was accompanied with the combination of 16 hours tempering and one-day storage and the addition of E_2 (Hemicellulase + Xylanase + fungal α-amylase) enzyme (86.25 g).

Table 13: Means of freshness (TVT) of pan-bread made from Australian wheat as affected by the second order interaction among storage time, tempering time and added enzyme.

Storage	Tempering time	Peak force (g)		
		E_0	E_1	E_2
1day	8h	188.5	123.0	119.5
	16h	151.1	136.1	86.2
	24h	169.2	141.6	120.7
3days	8h	709.9	584.5	586.4
	16h	620.7	660.2	467.2
	24h	593.6	415.0	414.2
5days	8h	1125.0	968.0	673.0
	16h	1060.6	913.5	673.0
	24h	1064.0	910.0	673.0
$L.S.D._{0.05}$		35.47		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Increasing tempering time, affected improved flour quality. Tempering to 15% moisture, provided better separation of mellowed endosperm from toughened bran and finally increased gluten strength. (Kweon, *et al*, 2009). Treating wheat kernels during tempering by enzymes, such as; cellulase, xylanase, and β-glucanase, had a positive effect on improving final products quality. Bread quality characters represented by volume, crumb and firming are major affected quality characters. Although of that, enzymes addition to tempering water has been less studied. The major objectives of enzymes use are improving milling and baking performance (Haros *et al*, 2002, and Yoo, *et al*, 2009). Hille and schooneveld-Bergmaus, 2004, stated that, the addition of cellulase and cellobiohydrolyase to fungal and bacterial endoxylauase enzymes improved the tempering process. Cellulases open and break down cellulase, which inter twined with the arabinoxylan polymer, resulted in helping the endoxylanase activity. Such synergistic effect had a positive influence on bread volume and crumb softness. The addition of combined enzymes cellulase, xylanase and pectinase to tempering water gave improvement in bread firmness (Yoo *et al*, 2009).

7. Sensory evaluation of pan-bread

Sensory evaluation of pan-bread included crust color, crumb color, flavor, texture, taste and overall acceptability. Texture was significantly affected by the interaction among tempering temperature × tempering time × added enzyme (Table 14). The highest significant values of texture were obtained when tempering temperature was 20 ℃ for eight hours tempering and adding (E_1) enzymes or when tempering temperature was 40 ℃ for 24 hours tempering and adding (E_2) enzymes, (8.000 and 8.000, respectively).

Table 14: Means of sensory evaluation of pan-bread of Australian wheat as affected by tempering water temperature, tempering time and added enzyme.

Tempering water temperature	Tempering time	Texture		
		E_0	E_1	E_2
20℃	8h	7.333	8.000	6.667
	16h	4.667	6.000	7.333
	24h	5.333	6.667	6.667
40℃	8h	5.333	7.333	7.333
	16h	4.667	6.667	7.333
	24h	7.333	5.333	8.000
$L.S.D._{0.05}$		0.165		

E_0: control, E_1: Hemicellulase + Xylanase, E_2: Hemicellulase + Xylanase + fungal α- amylase.

Best-fit equations that related grain properties of Australian wheat grade to tempering time, tempering water temperature and added enzyme and the out-come might be summarized as follow:

Stability R^2 =0.77

Y= -919.659 + 11.383[Test weight (kg/h)] + 5.095[Particle size index (psi %)] - 1.628[Tempering time] + 0.243[Tempering temperature] - 8.881[Tempering added enzyme].

Loaf volume R^2 =0.78

Y= 17956.161 - 212.978[Test weight (kg/h)] - 12.863[Particle size index (psi %)] + 18.246[Tempering time] +1.430 [Tempering temperature] + 102.621[Tempering added enzyme].

Acar *et al.*, 2019, postulated that kernel size and kernel hardness had an effect on flour yield rather than flour quality. They added that kernel hardness might determine the quality of end-product. The major objectives of enzymes use are improving milling and baking performance (Haros *et al*, 2002, and Yoo *et al*, 2009). Hille and Schooneveld-Bergmaus, 2004, stated that, the addition of cellulase and cellobiohydrolyase to fungal and bacterial endoxylauase enzymes improved the tempering process. Cellulases open and break down cellulase, which inter twined with the arabinoxylan polymer, resulted in helping the endoxylanase activity. Such synergistic effect had a positive influence on bread volume and crumb softness. Addition of xylanase enzyme to tempering water gave a positive impact on dough rheology characters and bread sensory evaluation attributes (Ahmad *et al*, 2013).

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تاثير زمن ودرجة حرارة التكييف و اضافة الانزيمات على محصول الدقيق ومواصفاته لرتبة القمح الاسترالى

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الملخص العربى

الأهداف الرئيسية للدراسة الحالية تهدف الى الربط بين الرتبة التجارية للقمح وكل من زمن الترطيب ودرجة حرارة مياه الترطيب والأنزيمات المضافة مع مياه الترطيب ومحصول الدقيق وخصائص الدقيق. زمن الترطيب تم تمثيله بثلاث مستويات هي، ٨ و ١٦ و ٢٤ ساعة ترطيب. استخدم مستويان من درجة حرارة مياه الترطيب هى ٢٠ و ٤٠ درجة مئوية. بالنسبة للأنزيمات فى مياه الترطيب وقد مثلها ثلاث مستويات: بدون معاملة أو مخلوط هيميسيلوليز + زيلاناز أو مخلوط هيميسيلوليز + زيلاناز + ألفا أميليز الفطري. سجلت أعلى نسبة بروتين (١٥,٢٧%) من المعاملة المؤلفة بين ٢٠ درجة مئوية لمياه الترطيب مع ١٦ ساعة زمن ترطيب. بينما سجلت أقل نسبة بروتين من التوليفات ٢٠ درجة مئوية لحرارة الترطيب و ٢٤ ساعة زمن الترطيب (١٤,٩٠%) أو ٤٠ درجة مئوية حرارة ترطيب مع ١٦ ساعة زمن ترطيب (١٤,٩٧%). هذا وسجلت أعلى نسبة بروتين مع توليفة المعاملات ١٦ ساعة ترطيب وعدم إضافة انزيمات (١٥,٢٣%)، بينما سجلت أقل نسبة بروتين مع توليفة معاملات ٢٤ ساعة ترطيب واضافة أي من (هيميسيلوليز + زيلاناز) أو (هيميسيلوليز + زيلاناز + ألفا أميليز الفطري). تحققت أعلى قيمة لنسبة الجلوتين ورقم السقوط (٣٤,٤٣% و ٥٧٨,٥ ثانية على الترتيب) عن الترطيب لمدة ١٦ ساعة وعدم إضافة أى انزيمات. سجلت أعلى قيمة معنوية لرقم السقوط مع حرارة مياه ترطيب ٢٠ درجة مئوية مع أي من فترات الترطيب ١٦ أو ٢٤ ساعة وعدم أضافة أنزيمات (٦٠٣,٠ و ٥٩٨,٣ ثانية على الترتيب). هذا وسجل أعلى قيمة لمعدل استخلاص معنوى لترطيب لمدة ٢٤ ساعة مع أضافة هيميسيلوليز + زيلاناز + ألفا أميليز الفطري (٧١,٦٠%). وفى المقابل فقد سجل أقل معدل استخلاص مع ٨ ساعات ترطيب ومخلوط أنزيم هيميسيلوليز + زيلاناز (٦٦,٠٠%). أعلى قيمة لرقم جودة الفارينوجراف معنوية سجلت مع توليفة معاملات ٢٠ درجة مئوية و ٨ ساعات زمن ترطيب و إضافة انزيمات هيميسيلوليز + زيلاناز (٤٣٠,٠ مم). بينما نتجت اقل قيمة من توليفة معاملات ٢٠ درجة مئوية و٨ ساعات و إضافة انزيمات هيميسيلوليز + زيلاناز + ألفا أميليز الفطري (٩٥,٠٠ مم). وقد نتجت أعلى قيمة لقوة العجين من توليفة معاملات ٢٠ درجة مئوية لحرارة مياه الترطيب و ٢٤ ساعة زمن ترطيب و اضافة خلطة انزيمات هيميسيلوليز + زيلاناز (٤٨٤ 10^{-4}جول) بينما سجلت أقل قيمة مع توليفة معاملات ٤٠ درجة مئوية و ١٦ ساعة زمن ترطيب وعدم أضافة انزيمات (٢٣٩ 10^{-4}جول). سجلت أعلى قيمة لوزن الرغيف عند زمن ترطيب ١٦ ساعة مع عدم أضافة انزيمات (٢٥٦,١ جرام).أعلى قيم لحجم الرغيف و الحجم النوعى سجلت مع زمن ترطيب ١٦ و ٢٤ ساعة و إضافة هيميسيلوليز + زيلاناز (١٥٢٠ أو ١٥١٨,٧ سم٣ و ٦,٩٠٣ أو ٦,٢٤٤ سم^3/ الجرام) لتوليفة المعاملات على الترتيب). سجلت أعلى قيمة معنوية لقوة الضغط مع توليفة معاملات: ٨ ساعة ترطيب و التخزين لمدة ٥ أيام و عدم أضافة اى انزيمات (١١٢٥ جرام). سجلت أقل قيمة لقوة الضغط مع ١٦ ساعة ترطيب ويوم واحد تخزين و إضافة انزيمات هيميسيلوليز + زيلاناز + ألفا أميليز الفطري (٨٦,٢٥ جرام). سجلت أعلى قيمة معنوية للقوام عند ٢٠ درجة مئوية

لمياه الترطيب و لمدة ٨ ساعات زمن ترطيب و أضافة انزيمات هيميسيلوليز + زيلاناز + ألفا أميليز الفطري او عند ٤٠ درجة مئوية لمياه الترطيب و لمدة ٢٤ ساعات زمن ترطيب و أضافة انزيمات هيميسيلوليز + زيلاناز + ألفا أميليز الفطري (٨,٠٠٠ أو ٨,٠٠٠ على الترتيب). يمكن تلخيص أفضل المعادلات التي تربط خصائص الحبوب من درجة القمح الأسترالي بزمن الترطيب ودرجة حرارة ماء الترطيب والإنزيم المضاف على النحو التالي:

Stability R^2 =0.77

Y= -919.659 + 11.383[Test weight (kg/h)] + 5.095[Particle size index (psi %)] - 1.628[Tempering time] + 0.243[Tempering temperature] - 8.881[Tempering added enzyme].

Loaf volume R^2 = 0.78

Y= 17956.161 - 212.978[Test weight (kg/h)] - 12.863[Particle size index (psi %)] + 18.246[Tempering time] +1.430 [Tempering temperature] + 102.621[Tempering added enzyme].