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Article

The Influence of Prebiotics on Wheat Flour, Dough, and Bread Properties; Resistant Starch, Polydextrose, and Inulin

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Abstract: The addition of prebiotics is one of the most important ways to improve the techno-functional properties of bread. In this study, the effects of resistant starch, polydextrose, and inulin on the wheat flour, dough, and bread properties were investigated. In farinography results, resistant starch significantly increased the development time (2:18) with a boosting effect, but polydextrose (1:48) and inulin (1:36) weakened the dough (P <0.05). Inulin, polydextrose, and resistant starch had the most effect on reducing water absorption (40, 43.2, and 48.9) respectively (P <0.05). According to extensography data, inulin had the best result in baking compared to other polysaccharides. In terms of baked breads, samples containing resistant starch had high moisture that could be due to starch gelatinization and moisture-retaining, which delays the staling process of bread. Inulin, polydextrose, and resistant starch prebiotic ingredients affected dough rheological properties and bread quality, and organoleptic characteristics, however, resistant starch was the best.

Keywords: prebiotics; resistant starch; inulin; polydextrose; farinography; extensography

1. Introduction

Prebiotics as non-digestible ingredients are digested by the colon's microflora and produce health-promoting compounds, such as various organic acids, short-chain fatty acids, and vitamins [1,2]. The following changes in the composition or activity of the intestinal microflora improve the health and well-being of the host by different mechanisms including; enhancing satiety and inhibition of obesity, regulating gut movements, preventing diarrhea and constipation, and reducing the deposition of harmful microorganisms, such as Bacillus, Salmonella, and Staphylococci in the colon [2,3]. Regular and prolonged use of prebiotics also regulates and stimulates the immune system and increases the body's resistance to various types of diseases [4-6]. Increasing the absorption of vital minerals and vitamins, reducing blood cholesterol and improving insulin sensitivity and positive effects on cancers, especially gastrointestinal cancers, and metabolic syndrome, are other beneficial effects of these functional compounds [7-10].

The European Food Standards Agency (EFSA) has set the dietary reference value for dietary fibers and prebiotics at 25 g per daily diet for an adult aged 18 years or over to maintain normal abdominal functions but acknowledges that higher levels of absorption are more beneficial [11]. The study of National Health and Nutrition Examination Survey (NHANES) found that 20 years and older Americans use only 61% of their apparent levels, while, official recommendations regarding the use of prebiotics have not been published and some researchers have come up with suggestions like 10 g fructo-oligosaccharides (FOS) and 7 g galacto-oligosaccharides (GOS) per day [12-14]. The prebiotic effect can be observed in low dosages, such as 2.5-5 g/day for resistant starch [15], 1-6 g/day for inulin [16], and 2-7.5 g/day for polydextrose [17].

Resistant starch is a carbohydrate that resists ingestion in the gastrointestinal tract, but ferments in the colon and acts as a prebiotic and feeds the probiotic microorganisms in the colon. Resistant starch is one of the most important prebiotic ingredients that can be used in preparing different kinds of bakery products. The enrichment of bread crumbs with resistant starch is gaining importance and can be found as an impactful approach to improve consumer healthiness over nourishment [18,19]. Also, inulin (fructan) is fermented via the gut microbiome and is considered a prebiotic ingredient. various studies have displayed that using inulin as a prebiotic improves the nutritious and healthy properties of the different kinds of breads, nevertheless, it has not shown any strong adverse impacts on rheological and organoleptic characteristics of the breads [20-22]. Resistant starch and inulin are examples of potential prebiotics fulll filling this criterion which can also change farinography and extensography characteristics of dough [18,23,24]. Polydextrose is a kind of carbohydrate used as a prebiotic, and also used as a sweetener and to improve the texture of food products [25,26]. The impacts of this compound have not been extensively studied in bread or other similar products. Therefore, it is interesting to study the effects of polydextrose on the rheological and sensory characteristics of bread and compare it with the resistant starch and inulin.

According to the advice of experts, the type of prebiotics used in products that will be heated (>90 °C), such as pasta or other bakery products, should be resistant to high temperatures and not melted during the preparation processes [27]. It also should not lead to any undesirable taste and texture in final products, i.e. it is required to be consumer-friendly in terms of appearance and color [28,29]. The other point that motivated us to work on in this study was the lack of some essential amino acids especially lysine, and also threonine and tryptophan, in wheat flour downgrading the nutritional value of bread. Wheat flour has been enriched by whey that contains a lot of essential amino acids. Adding a small amount of whey can significantly improve the protein quality of wheat flour [30-33].

So far, there has been no study comparing the effects of these three prebiotic polysaccharides (resistant starch, inulin, and polydextrose) on the farinography and extensography characteristics of the resulting dough, and in particular the characteristics of the final bread produced. In this study, three different samples of prebiotic flours including inulin and whey powder, polydextrose and whey powder and resistant starch and whey powder have been evaluated, regarding their characteristics and the features of the produced dough and bread from these samples. The purpose of this study was to investigate which of the prebiotic compounds has the best effect on the physicochemical properties of flour and dough and the physicochemical and sensory properties of baked breads and is accepted for industrial production.

2. Materials and Methods

2.1. Formulation of prebiotic wheat flours

Commercially soft white flour was provided from Maragheh Flour Factory (Altin Flour, Maragheh, Iran). In a special mixer (YAYANG, China), the experimental samples were prepared according to **Table 1**. as 5% by weight of inulin (PYSON CO. LTD. China) + 5% by weight of whey powder (Zarin Laban Pars Company, Iran) (In sample), 5% by weight of polydextrose (PYSON CO. LTD. China) + 5% by weight of whey powder (PD sample), and 5% by weight of resistant starch (Hi-maize 260, National Starch, USA) + 5% by weight of whey powder (RS sample) each added to the flour mixture and completely mixed and homogenized. The control sample (Blank) was from the same flour with 5% by weight of whey powder but without any prebiotic ingredient addition. Regarding the fact that whey powder was used in all groups, the results were only attributable to prebiotic compounds (In, PD, and RS samples).

Table 1. Formulation of prebiotic wheat flours as experimental samples.

Sample	Prebiotic content (g- %)	Whey content (g, %)	Flour content (g, %)	Total content (g, %)
RS	50- 5	50- 5	1900- 90	2000- 100
PD	50- 5	50- 5	1900- 90	2000- 100
In	50- 5	50- 5	1900- 90	2000- 100
Control	0- 0	0- 0	2000- 100	2000- 100

RS= Samples contain resistant starch, PD=Samples contain polydextrose, In= Samples contain inulin.

2.2. Physicochemical characterization of flours

The quality of the control and formulated prebiotic flours were tested using American Association of Cereal Chemists (AACC) routine methods such as moisture (AACC, 16), ash (AACC, 08-01), protein (AACC, 46-12), wet and dry gluten, gluten index (AACC, 38-12A), Falling number (AACC 56-81B), and Zeleny test (AACC 56-60) based on the sample's initial weights [34].

2.3. Dough preparation and baking bread

A uniform method for the preparation of dough for bread-making was applied. The basic dough (control dough) was prepared from 2000 g flour consisted of salt (20 g), sugar (40 g), compressed yeast (60 g), and the volume of water required getting to 500 BU of consistency via the farinograph (Section 2.4) [35,36]. Wheat flour was mixed well with all prebiotic treatments according to **Table 1**. Bread doughs were formulated by blending all elements and fermented for 12 min; then dough portions (100 g) were divided, hand-moulded and sheeted. Doughs were proofed at 32 °C and 85% humidity up to optimal volume growth and baked at 260 °C for 17 min [37,38]. At the end of the baking, the bread was kept at room temperature for some time to cool and then packed in polyethylene bags for sensory testing.

2.4. Farinography tests

All samples of separate doughs (RS, In, and PD samples) were calculated on a flour dry weight basis and were assessed according to the AACC 54-21 method [34]. Resistant starch, polydextrose, and inulin in a dry powder form were first mixed well with the wheat flour into the mixing bowl (300 g) of the farinogragh (YUCEBAS MAKINE, Turkey, with 300 g dish) that was connected with a circulating water pump and a thermostat operating at 30±0.2 °C. The parameters of water absorption percentage, dough development time (DDT), dough stability, mixing tolerance index (MTI), and farinograph quality number (FQN) were determined accordingly.

2.5. Extensography tests

At first, all samples of separate doughs (RS, In, and PD samples), were each prepared in the 300 g mixing bowl of the farinograph. Water and salt were then added to produce the dough samples with a constancy of 500 BU, followed by 5 min of blending. A test quantity (150 g) was formed into a sphere, formed into a cylinder, and clamped into the fermenting cabinet. After 45, 90, and 135 min reaction times in the fermenting cabinet at 32 °C, each dough piece was overextended in the extensograph (YUCEBAS MAKINE, Turkey) device via a hook until rupture according to the AACC 54-10 method

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[34]. Extensograph equipment gave the extensibility (E), the ratio max., maximum resistance, and energy.

2.6. Determination of baked bread properties

To measure the moisture content of baked loaves of bread, the 44-16 AACC method was carried out on day 1 [34]. To determine the bread volume, 2 h after baking, the AACC 10-05 method was applied, and finally, the specific volume of the loaf was determined by the following equation [34]. Tests of crumb firmness were performed according to the AACC method 74-09 [34].

$$\textbf{Specific volume (cm}^3\textbf{/g) = loaf volume/loaf weight} \quad (1)$$

2.7. Sensory analysis of produced bread

The organoleptic characteristics of final breads prepared from control and prebiotic flours were carried out by a panel of 12 trained people (20-40 years old, non-smokers) at room temperature. The extreme marks for each factor were: appearance 10, color 10, chewiness 15, crust 15, texture 15, aroma 15, and flavor/taste 20 [15].

2.8. Statistical analysis

The sample size was estimated based on the moisture percentage of bread loaves. Assuming the power of 80%, 95% confidence interval, and an effect size of 2 and the standard deviation of 0.8, the calculated sample size was 3 per group [39].

All tests were performed with three replications. The data were presented using mean (SD). Shapiro-Wilk test carried out to test the normality of data. According to the results, all the variables were normally distributed. Subsequently, three experimental groups were compared using One-Way ANOVA followed by Bonferroni post hoc tests. $P<0.05$ was considered a significant level. The test was not performed for moisture, Zeleny I, Zeleny II and pH, because the standard deviations of all the groups were 0.

3. Results and discussion

3.1. Quality of formulated prebiotic wheat flours

The results of the physicochemical quality of the control and formulated prebiotic flours are shown in **Table 2**. The amount of ash in the control sample was significantly lower than other samples ($P<0.01$) because of the addition of prebiotics. High levels of ash indicate high levels of minerals and the high nutritional value of prebiotic flours. Minerals also play an important role in increasing gluten quality during dough preparation by improving the performance of the gluten network. On the contrary, the moisture content in the control sample was higher than the rest of the samples. Protein content in the tested flours was slightly lower than in the control sample due to gluten dilution by the addition of prebiotics. Also, Zeleny I and II in the control sample was higher than all of the prebiotic samples. Reducing the percentage of humid/ wet gluten (H gluten) and dry gluten (D gluten) in samples containing prebiotics compared to the control sample is similar to protein percentage due to the addition of gluten-free materials and the reduction of total gluten content. However, comparing the gluten index among the tested samples, especially in the case of inulin sample, showed that with a significant increase in the quality of gluten and a reduction in gluten content, strengthening the gluten network and improving the performance of flour and dough by storing the gases during the fermentation process have been done. The resulting changes in the Falling number are not significant due to the fact that they aren't outside the 200-400 range and are considered to be desirable in all four samples.

Table 2. Physicochemical characteristics of the dough prepared from different flour samples (Mean±SD).

Sample	Total ash (%)	Moisture (%)	Protein (%)	pH	ZelenyI (ml)	ZelenyII (ml)	H Gluten	D Gluten	Gluten Index	Falling Number (s)
PD	0.94 ± 0.01[a]	12.9 ± 0.00	9.10 ± 0.00[a]	6.12 ± 0.00	18.00 ± 0.00	21.00 ± 0.00	21 ± 0.42[a]	7.00 ± 0.00[ab]	92.85 ± 0.49[ab]	322 ± 15.56
In	1.00 ± 0.07[a]	13.1 ± 0.00	9.25 ± 0.95[a]	6.21 ± 0.00	21.00 ± 0.00	21.00 ± 0.00	18.5 ± 0.5[b]	6.45 ± 0.05[b]	97.05 ± 0.25[a]	343.00 ± 8.00
RS	1.04 ± 0.06[a]	13.5 ± 0.00	10.25 ± 0.21 [ab]	6.12 ± 0.00	18.00 ± 0.00	22.00 ± 0.00	24 ± 0.28[c]	7.9 ± 0.14[a]	87.65 ± 6.29[ab]	333 ± 1.41
Control	0.70 ± 0.02[b]	14.00 ± 0.00	11.10 ± 0.42[b]	6.10 ± 0.00	23.00 ± 0.00	28.00 ± 0.00	26.3 ± 0.28[d]	8.55 ± 0.35[c]	87.3 ± 2.83[b]	354.00 ± 18.38
P Value	**0.007**	-	**0.015**	-	-	-	**<0.001**	**<0.001**	**0.024**	0.242

PD=Samples contain polydextrose, In= Samples contain inulin, RS= Samples contain resistant starch.

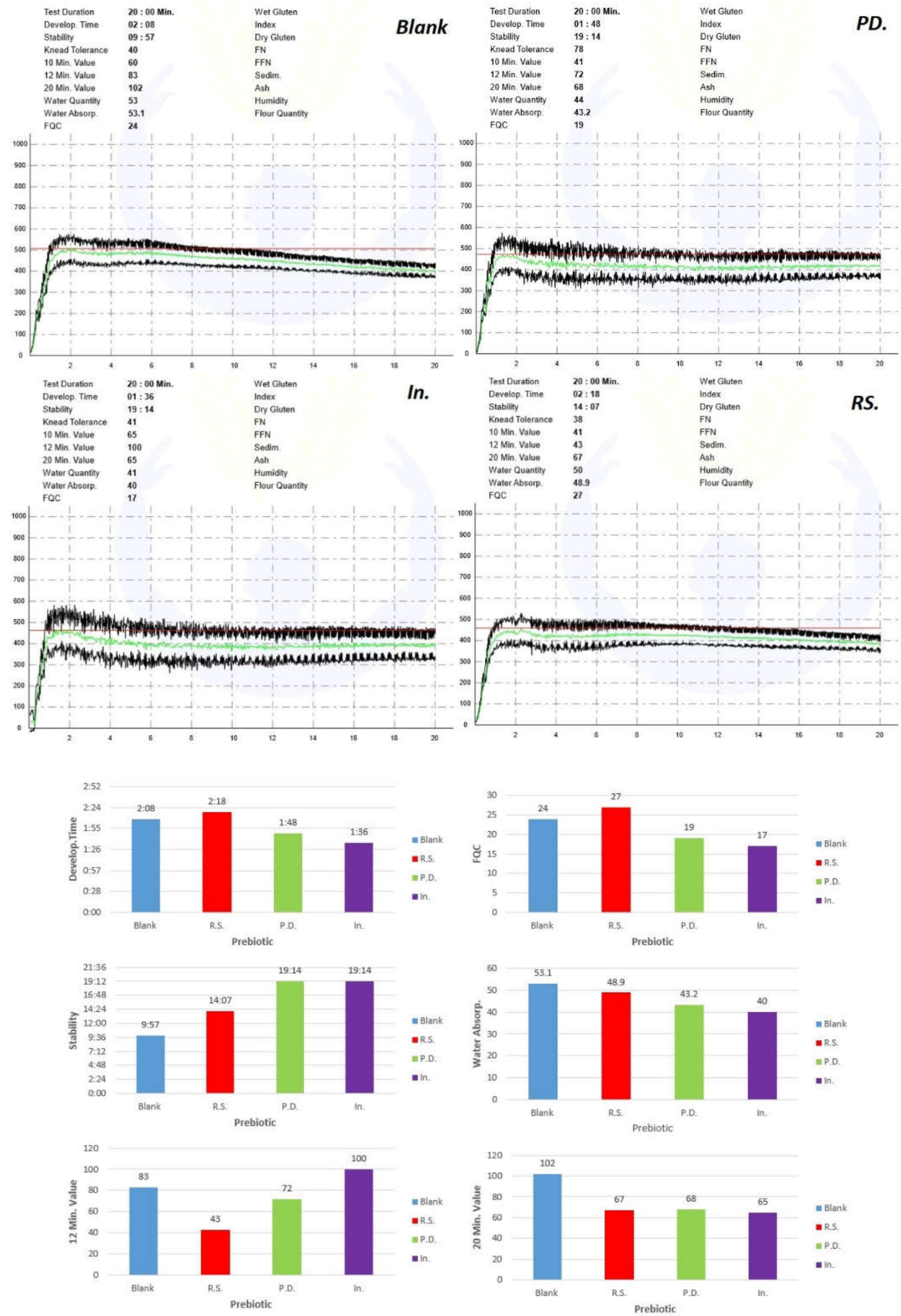
Significant *P Values* are marked in bold.

Different lower-case letters indicate statistically significant differences ($P < 0.05$) between experimental groups.

3.2. Farinograghy results

The results of farinography tests and analyzing curves are shown in **Fig. 1**. The resistant starch significantly increased the development time and had a boosting effect, but polydextrose and inulin weakened the dough. In the case of stability, all the ingredients added to the flour have played a boosting role in the dough as the dough persists. The amount of loosening of the dough was reduced after 12 min by adding resistant starch and polydextrose (dough reinforcement) and increased with the addition of inulin (weakening of the dough). The amount of loosening of the dough after 20 min was reduced by adding all three ingredients (the effect of dough reinforcement through increased stability). In terms of water absorption, inulin, polydextrose, and resistant starch had the most effect on reducing water absorption, respectively. Considering that inulin had the highest impact on the water absorption percentage, as a result of increasing inulin consumption, the percentage of flour water absorption decreases. This can be attributed to the composition of inulin i.e., fructose-polysaccharides, which is in accordance with the findings of Mohtarami et al. (2015), Wang et al. (2002), and Peressini and Sensidoni (2009) [30,40,41].

Farinography quality number (FQN) can be considered as a combination of different parameters. According to the results, and as seen in **Fig. 1**, resistant starch, polydextrose, and inulin had a negative effect on the characteristics of the dough in terms of farinography. It is believed that gluten creates specific dough structures and plays a significant role in dough and bread characteristics.


Blank
Test Duration 20 : 00 Min.
Develop. Time 02 : 08
Stability 09 : 57
Knead Tolerance 40
10 Min. Value 60
12 Min. Value 83
20 Min. Value 102
Water Quantity 53
Water Absorp. 53.1
FQC 24
Wet Gluten
Index
Dry Gluten
FN
FFN
Sedim.
Ash
Humidity
Flour Quantity
PD.
Test Duration 20 : 00 Min.
Develop. Time 01 : 48
Stability 19 : 14
Knead Tolerance 78
10 Min. Value 41
12 Min. Value 72
20 Min. Value 68
Water Quantity 44
Water Absorp. 43.2
FQC 19
In.
Test Duration 20 : 00 Min.
Develop. Time 01 : 36
Stability 19 : 14
Knead Tolerance 41
10 Min. Value 65
12 Min. Value 100
20 Min. Value 65
Water Quantity 41
Water Absorp. 40
FQC 17
RS.
Test Duration 20 : 00 Min.
Develop. Time 02 : 18
Stability 14 : 07
Knead Tolerance 38
10 Min. Value 41
12 Min. Value 43
20 Min. Value 67
Water Quantity 50
Water Absorp. 48.9
FQC 27
Develop.Time
2:08
2:18
1:48
1:36
FQC
24
27
19
17
Stability
9:57
14:07
19:14
19:14
Water Absorp.
53.1
48.9
43.2
40
12 Min. Value
83
43
72
100
20 Min. Value
102
67
68
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Blank
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Prebiotic

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Figure 1. a) Original farinograph curves of wheat flour (Blank=control) and wheat flour enriched with polydextrose (PD), inulin (In), and resistant starch (RS); b) Analyzing farinograph curves of wheat flour (Blank) and wheat flour enriched with polydextrose (PD), inulin (In), and resistant starch (RS).

Hence, the dilution of gluten via added fibers causes the breaking of the starch-gluten network and a reduction of dough stability [42]. Consequently, the replacement of a certain portion of wheat flour with some materials like inulin, polydextrose, and resistant starch leads to a decrease in protein content. In contrast, some investigations indicated that particular hydrocolloids and fibers improved rheological characteristics of wheat flour, for instance, water absorption, dough stability, and dough development time [15,43,44]. The dilution of gluten by fibers lonely cannot describe all of the alterations in the addition of fiber to wheat flour. The effect of various kinds of fibers on dough characteristics can be described by reactions between gluten protein of flour and fibers [15,44].

3.3. Extensography results

The results of extensography tests and analyzing curves are shown in **Fig. 2**. Regarding the results of extensography, it can be concluded that the added ingredients (resistant starch, polydextrose, inulin) to the normal flour played the role of oxidizing or reinforcement in the flour. By increasing the strength of the dough and reducing the extensibility, it increases the ratio number and the energy of the dough. Among the added ingredients according to the energy curve, inulin was predicted to have the best result in baking compared to other samples, but it can happen if we consider the absorption of water in the dough-making process. Probably, inulin due to its physicochemical properties reduces proteolytic degradation and thus improves protein flour behavior. In this regard, Brennan et al. (2004), and Karolini-Skaradzinska et al. (2007) reported the raise in the energy needed for dough transformation by increasing the inulin level [45,46]. It seems that inulin, because of its specific physicochemical properties, reduces proteolytic degradation and thus improves the protein behavior of the flour.

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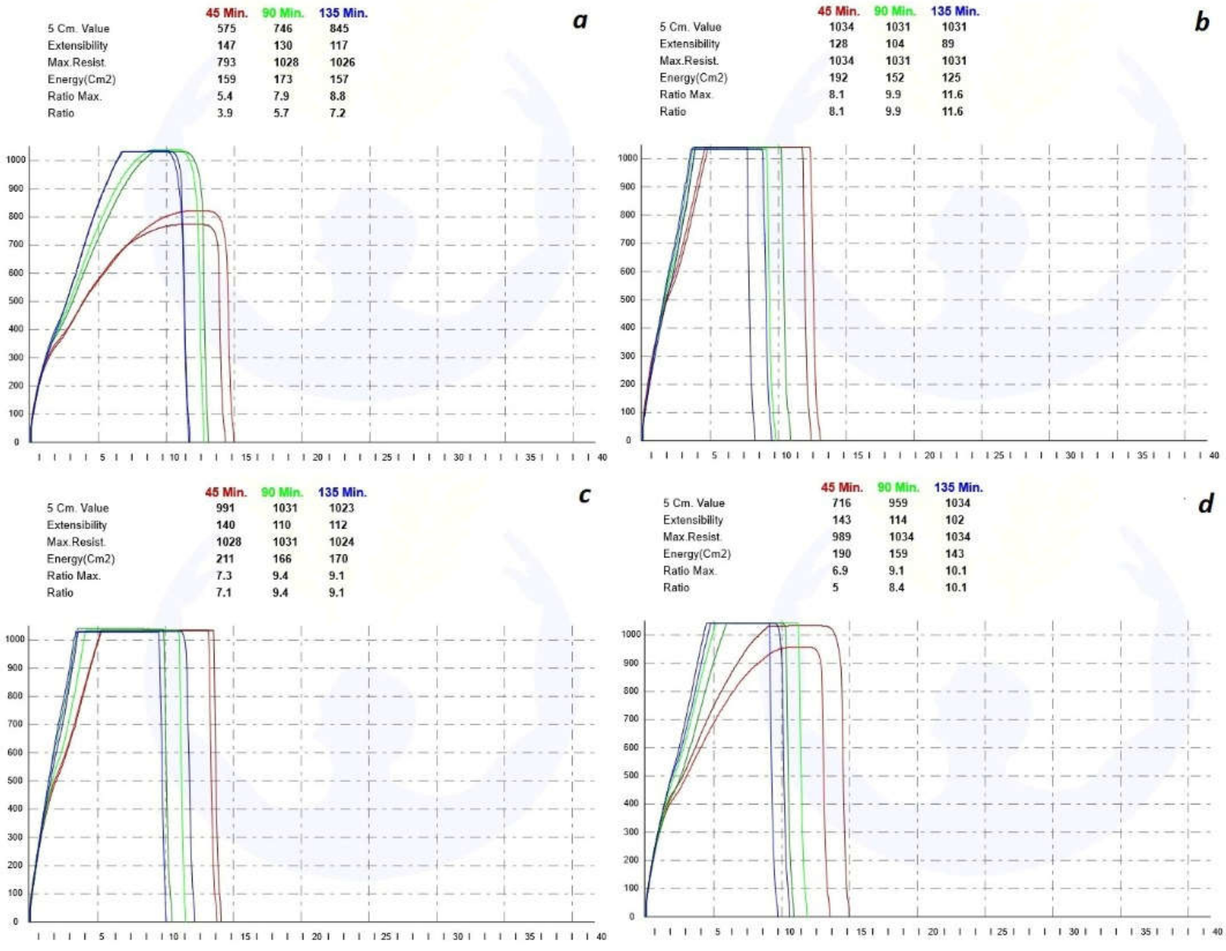

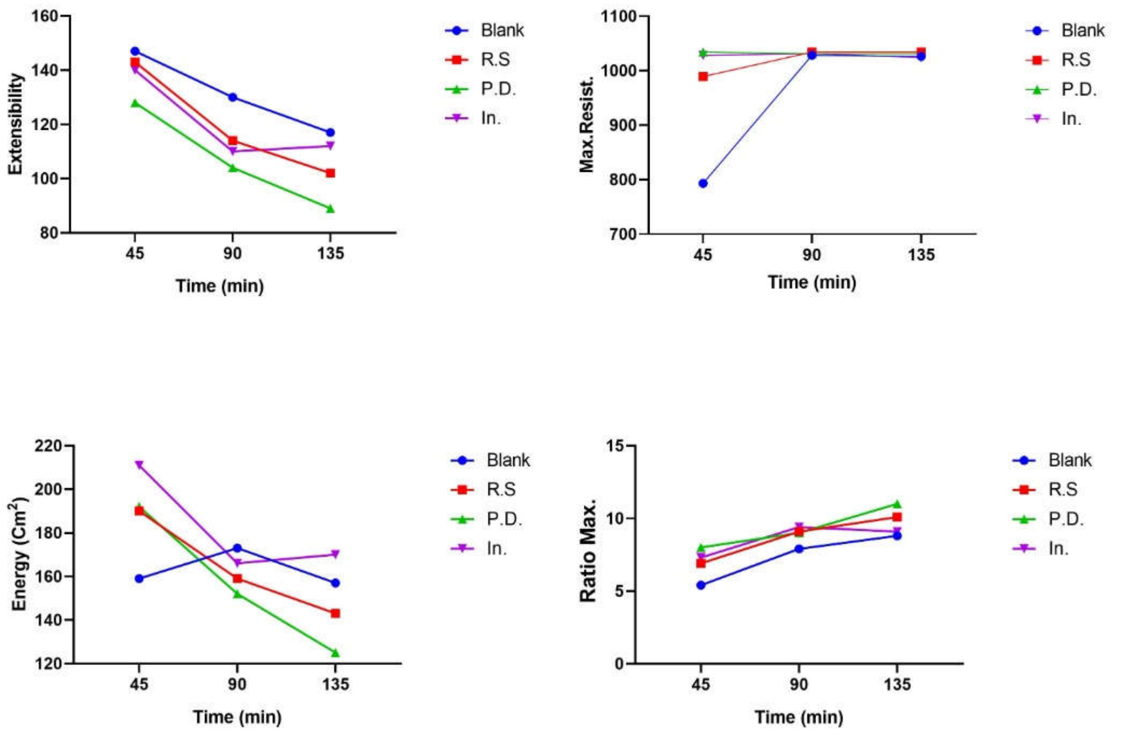

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Figure 2. a) Original extensograph curves of wheat flour (Blank=a) and wheat flour enriched with polydextrose (b), inulin (c), and resistant starch (d); b) Analyzing extensograph curves of wheat flour (Blank) and wheat flour enriched with polydextrose (PD), inulin (In), and resistant starch (RS).

3.4. Quality of prebiotic baked breads

The results of moisture content, crumb firmness, and specific volume of cooked breads (two hours after cooking) are shown in **Table 3**. Among the tested samples, the highest and the lowest moisture content was seen in the loaves containing resistant starch and inulin (RS and In samples) respectively. The moisture content of the loaf contained inulin (In sample) was the least and largely similar to the control sample (Blank). In various studies, crumb firmness was reported with the addition of different hydrocolloids [15,47,48]. In this study, the bread fortified with resistant starch (RS sample) exhibited the highest firmness compared with other samples (In, PD, and, Control samples). The crumb firmness of loaf containing inulin (In sample) was smaller than the control sample. In fact, the level of crumb hardening is affected via water content and it is considered as one of the most significant factors for bread staling.

Table 3. Moisture content, crumb firmness, and specific volumes of bread loaves (Mean±SD).

Sample	Moisture content (%)	Crumb firmness (N)	Specific volume (cm^3/g)
RS	38.30±0.80^{b}	1.02±0.08^{d}	3.45±
In	34.99±0.11^{a}	0.45±0.04^{a}	2.99±
PD	36.54±0.18^{a,b}	0.85±0.09^{c}	3.25±
Control	35.09±0.25^{a}	0.70±0.02^{b}	2.94±

RS= Samples contain resistant starch, In= Samples contain inulin, PD=Samples contain polydextrose.
Same small letters within the same column are not significantly different ($p > 0.05$).

Various studies have shown that the type and amount of prebiotic compounds seem to affect the rheological characteristics of the dough and so have an impact on the specific volume of the fortified bread loaves [15,49,50]. In this study, the specific volume of the bread fortified with resistant starch (RS sample) was the highest and similar to the sample containing polydextrose (PD sample). Also, the specific volume of the bread fortified with inulin (In sample) was the least and largely similar to the control sample. According to **Table 3**, it was observed that the effect of resistant starch on the resulting breads was somewhat higher. This sample contains high moisture that could be due to starch gelatinization and moisture-retaining, which delays the staling process of bread. On the other hand, this could be attributed to bread crunching and to some extent a better spread of bread (the ratio of volume to bread weight is greater than the rest of the samples, see **Table 3**) which also affects sensory properties. This kind of bread spreads in the mouth earlier, so the taste of the food can be better tasted (as if bread is finished earlier than food in the mouth).

3.5. Sensory properties of final breads

Sensory features were evaluated by the numerical scoring method. All tests were performed in triplicate and the results were reported as average and standard deviation (**Table 4**). Based on the results, among the produced bread samples (**Fig. 3**), those containing resistant starch (RS sample) had the highest score in all organoleptic items including total acceptability, with the exception of the appearance and color. On the other hand, breads containing polydextrose (PD sample) had the lowest score for organoleptic indices and total acceptability. In examining the appearance and color of the samples, except for the control sample, in all samples, brown spots can be observed on the bread surface which is an undesirable feature. It seems that these spots are caused by the nonuniform mixing of the added prebiotic compounds into the flour that are nodded in the surface of the dough and burned briefly under the heat. In other words, the reason for the change in the color of the breads during baking, which is caused by the baking temperature and the phenomenon of non-enzymatic browning [33], was uniform everywhere on the surface of the control breads, but on the surfaces of the other tested breads that contained prebiotics, there were scattered brown spots darker, which indicates the occurrence of more intense browning phenomenon.

Table 4. Sensory scores (Mean±SD) of control bread and loaves of bread supplemented with polydextrose (PD sample), inulin (In sample), and resistant starch (RS sample).

Sample	Appearance	Color	Chewiness	Crust	Texture	Aroma	taste	Total Acceptability
RS	7.5±0.0	8.5±0.7	14.75±0.3	14.75±0.3	12.5±0.7	15.0±0.0	17.5±0.7	**90.5±0.71**
PD	7.0±0.0	6.5±0.7	13.5±0.7	12.0±1.4	11.5±0.7	11.0±1.4	15.0±0.0	**76.5±3.5**
In	7.7±0.3	9.5±0.7	14.25±0.3	11.0±1.4	12.25±0.3	14.0±0.0	16.5±0.7	**85.25±3.2**
Control	10.0±0.0	10.0±0.0	14.25±0.3	13.0±0.0	12.0±0.0	14.0±0.0	16.0±0.0	**89.25±0.35**

RS= Samples contain resistant starch, PD=Samples contain polydextrose, In= Samples contain inulin.

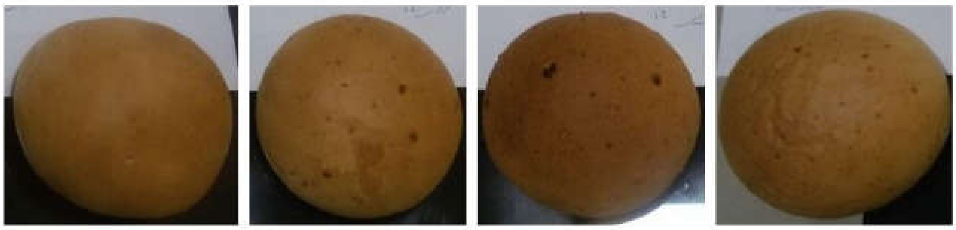

Figure 3. Loaves of control bread and loaves of bread supplemented with inulin (In), polydextrose (PD), and resistant starch (RS). Loaves arranged from left to right Blank/Control, In, PD, and RS samples respectively.

Because it was an experimental study, the study condition was completely under control and was similar for all the experimental groups rolling out the probability of selection bias. Since all the objective measurements were done with a single standard method and similar devices and personnel, the chance of measurement bias was also very low. The only parameters which were measured subjectively were the organoleptic characteristics. In this case, we kept the participants unaware of the types of samples to reduce the measurement bias.

4. Conclusions

According to the results of this study, inulin, polydextrose, and resistant starch prebiotic ingredients affected dough rheological properties and bread quality, and organoleptic characteristics. It can be concluded that among all the studied prebiotic polysaccharides, resistant starch was the best compound that may be used for formulation and producing prebiotic bread. It can also raise the shelf life and reduce the staling of the produced bread. According to the results of this article and many similar published articles, in most studies, the use of prebiotic compounds does not have a very adverse effect on the quality of bread, although various other investigations should be expanded to find the best prebiotic or the best prebiotic compound. In the near future, we will see the use of a wide range of prebiotic compounds in the production of various foods, especially breads, which are the main food of the people of the world.

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S.M.J; visualization, H.P and S.A.I.; supervision, S.M.J; project administration, H.P.; funding acquisition, H.P and S.M.J All authors have read and agreed to the published version of the manuscript."

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