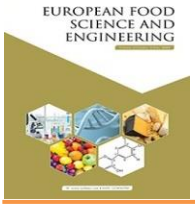

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The effects of high molecular weight glutenin subunits in wheat flours on soft- and hard-dough biscuit products quality

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ABSTRACT

In this study, the effects of HMW-GS on various quality characteristics of certain soft wheat biscuit products were investigated. Results of the study showed that subunit 5x + 10y in the *Glu-D1* locus increased the gluten strength of a flour and, consequently, negatively affected the physical and sensory properties of the resulting soft-dough biscuit products made with that flour. While product results suggested that the wheat genotypes carrying the allele 1 in *Glu-A1* should be selected with care, in terms of biscuit sensory properties, the combination of the null allele in *Glu-A1* and subunits 7 in *Glu-B1* and 2x + 12y in *Glu-D1* can lead to increased success of wheat breeding programs aimed at flours preferred for soft-dough biscuit products. For hard-dough biscuit products, subunit 2* can be used as a marker, in order to eliminate those soft wheat lines having too-high gluten strength. The combinations of HMW-GS 1, 7x + 9y, and 5x + 10y, along with the 1B/1R wheat-rye translocation, were considered to result in flours for production of hard-dough group biscuits. This study suggested that the most suitable HMW-GS combinations considering wheat-rye translocations could be used for selection purposes in breeding programs for soft wheat baked products.

1. Introduction

Wheat gluten (gliadins + glutenins) is the key protein responsible for the processing quality of wheat flours used to produce various bakery products (e.g. leavened breads and biscuits) that frequently make up a common part of our daily diet (Uthayakumaran & Wrigley, 2017). The glutenin proteins comprise high molecular weight (70000-90000 Da) and low molecular weight (20000-45000 Da) glutenin subunits (Ma *et al.*, 2019). The high molecular weight glutenin subunits (HMW-GS), which are polymeric proteins capable of inter-chain disulphide bonding (Nazrul *et al.*, 2003), are encoded by three allelic genes, which are localized on the long arm of chromosome 1 (1AL, 1BL, and 1DL), and these loci are identified as *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively (Payne *et al.*, 1987). Although the HMW-GS generally constitute only 10-15% of the storage proteins in a wheat grain, they are the proteins mainly responsible for the variability in the gluten strength of flours and the resulting viscoelastic properties of wheat flour doughs (Zhao *et al.*, 2020). Consequently, the HMW-GS proteins are mainly associated with the sensory and physical properties related to the final product quality of various types of wheat flour-based bakery products (Weegels *et al.*, 1996). The specific composition of the HMW-GS is widely used for selection purposes, in several stages from early to advanced generations, in wheat breeding programs (Morgounov *et al.*, 2013).

Between the subunits encoded in *Glu-D1*, the subunit 5x + 10y was shown to be associated with high gluten strength (Ng and Bushuk, 1987; Payne et al., 1987), attributed to carrying more cysteine residues (Kasarda, 1999), whereas the subunit 2x + 12y was reported to be related to and responsible for weaker and extensible doughs (Gianibelli *et al.*, 2001). Generally, in the other two loci, while it was determined that the subunits 1 and 2* in *Glu-A1* and 17x + 18y and 7x + 8y in *Glu-B1* were also associated with high gluten strength, the null and 7 alleles were found to be related to lower gluten strength (Ng & Bushuk, 1987; Payne *et al.*, 1987). Payne *et al.* (1987) suggested a quality score, in order to identify favorable and unfavorable subunits contributing to gluten strength, based on SDS sedimentation and alveograph W values. Accordingly, 1, 17x + 18y, and 5x + 10y HMW-GS located on different loci had the highest scores, while null, 7, 6x + 8y, 2, 3, and 4x + 12y had the lowest scores, in terms of bread-making quality. This scoring system brought considerable convenience to genetic selection in many wheat breeding programs (Kiszonas & Morris, 2018).

While the subunit 5x + 10y was used in selecting high bread-making quality (Bushuk, 1998), the wheat genotypes carrying the HMW-GS 2x + 12y in *Glu-D1* were evaluated as delivering superior biscuit-making quality (Finney & Bains, 2002). In various soft wheat breeding programs, in the USA, the subunit 5x + 10y was determined to result in too-strong gluten, while 2x + 12y was associated with desirably weaker gluten strength (Anon., 2017). It was reported that wheat

genotypes carrying the null allele (from Chinese spring wheat) had better biscuit-making quality than ones carrying the subunits 1 and 2* (from *T. monococcum*) in the *Glu-A1* loci (Tranquilli *et al*., 2002). However, a positive correlation was found between biscuit product diameter and subunit 1, and a negative correlation with subunit 2* (Hou *et al*., 1996). The Gerek79, Kırgız95, Sivas 111/33, and Sertak52 wheat varieties, used in the Turkish biscuit-making industry, had the HMW-GS compositions of 2*, 7x + 8y, and 2x + 12y (Demir *et al*., 1998). Besides the desired HMW-GS, soft wheat genotypes carrying the 1BL/1RS and 1DL/1RS wheat-rye translocations were reported to be a desirable option for improving soft wheat quality in certain breeding programs (Anon., 2017; Kim *et al*., 2005). While hard bread-wheat cultivars with strong and medium-strong gluten are typically classified for bread-making, flours from soft wheat cultivars with weak gluten, which produce more extensible doughs, have shown potential for use in making various types of biscuits or cookies (Guzman *et al*., 2016a; Kweon *et al*., 2014; Slade & Levine, 1994). Generally, a hard-dough biscuit (e.g. "Tea Biscuit") is a sheeted-dough biscuit with an open structure of layers and a smooth and shiny surface. It is produced from extensible doughs having well-developed gluten and relatively higher amounts of water, and less fat and sugar are used for such hard-dough biscuit production. In contrast, short-type creamy doughs, containing higher amounts of fat than water, are extruded through nozzles and then cut with a guillotine onto a belt, to produce different forms of soft-dough biscuit products (Karaduman, 2020).

This study aimed to evaluate the effects of HMW-GS and wheat-rye translocations on hard- and soft-dough biscuit flours and their resulting product-quality properties, and to suggest the most suitable HMW-GS combinations that could be used for selection, or elimination, purposes in breeding programs targeting soft wheats for specific baked products. Since determining subunits of LMW-GS is more time-consuming, difficult, and costly, in this study, the effectiveness of rapid assessment in soft wheat breeding programs with HMW-GS only was evaluated.

2. Materials and methods

2.1. Materials

Eleven soft winter bread-wheat lines were used in this study. The lines coded EK7, ES18, EK21, ES23, and EK24 had been developed for soft-dough biscuit products, while those codedES1, EK4, ES9, ES11, EK16, and ES7 had been developed for hard-dough biscuit products. The EK4, EK7, EK16, EK7, EK21, and EK24 lines were grown in seven locations under rainfed conditions, while the ES1, ES9, ES7, ES18, and ES23 lines were grown in five irrigated locations during the 2015-2016 and 2016-2017 crop seasons.

2.2. Technological quality traits

In both crop years, technological quality analyses were done on the flours from the eleven soft wheat lines grown at seven locations under rainfed conditions and five locations under irrigated conditions. A Labofix-90 mini cleaner (Brabender, Duisburg, Germany) was used to clean the wheat samples, which were then tempered to 14.5% moisture over 12 hours, and then milled to refined white flours on a laboratory milling machine (Model CD1, Chopin Technologies, Paris, France). A Zeleny sedimentation volume (ZSed) test was conducted according to ICC Standard Method 116/1 (ICC, 2011). A modified small-scale version of the standard solvent retention capacity (SRC) method AACC 56-11 (AACC 2010; Kweon et al. 2011) was conducted, using four solvents: water, sodium carbonate, sucrose, and lactic acid (Guzman et al., 2015). Starch damage was determined by an amperometric method, using the Chopin SDMatic, according to ICC Standard Method 172 (ICC, 2011). The amylolytic (α-amylase) activities of the flours were determined according to AACC Method 56-81.04, using a falling number tester (Perten-Falling Number) (AACC, 2010). Wet gluten, dry gluten, and gluten index analyses were performed according to AACC Method 38-12.02, using a Perten Glutomatic-2200 (AACC, 2010).

2.3. Biscuit-baking, physical, and sensory evaluations of products

In both crop years, hard- and soft-dough biscuits ("petit beurre" and "baby-type") were produced from the eleven flours mentioned in section 2.2. All biscuits were prepared in duplicate, following the Eti Food Industry and Trade Company commercial formulations. Biscuit production and sensory evaluations were done according to the specifications and references of the Eti Food Industry and Trade Company Research Center, Analytical Laboratory Section. The petit beurre or "tea biscuit" dough formula contained 90 g water, 100 g hydrogenated vegetable oil, 100 g whole milk, 200 g sucrose, 90 g invert sugar syrup, 16 g baking powder, and 6 g salt, based on 1 kg of flour. In petit beurre production, fast-speed, long-time mixing was done in one stage in a Z-type horizontal double-arm mixer. In order to achieve and maintain a dough temperature of 40-45 ºC, a cooling-jacketed mixer was used. The dough was rested for 18 minutes after mixing, and then baked on knitted-wire trays in a laboratory-type convection oven at 220 ºC for 5 minutes. The baby-type biscuit dough formula contained 240 g water, 280 g hydrogenated vegetable oil, 60 g wheat starch, 50 g whole milk, 400 g sucrose, 5 g baking powder, and 110 g whole egg, based on 1 kg of flour. In baby-type biscuit production, slow-speed, short-time mixing was done in two stages. In order to achieve and maintain a dough temperature of 18-24 ºC, cold water was used to make the dough. The dough was rested for about 20 minutes. Biscuits were produced by extruding the dough through nozzles and cutting it with a guillotine on to a belt, then baking the dough on flat steel trays in a laboratory-type convection oven at 210 ºC for 6 minutes. Both final biscuit products were packed, for later sensory evalution, after an hour of cooling to room temperature. Measurements of the width, length, and thickness of the biscuits, compared with references, were made an hour after baking, using a digital caliper. Biscuit moisture content analyses were done using a Sartorius MA45 Fast Infrared Analyzer at 140 ºC. Using a Stable Micro Systems TA Texture Analyzer Hd Plus, the breaking force of the biscuits, in g-f with a 30 kg-f load cell, was measured with a Three Feet P50 probe. These measurements were made at the center of each biscuit sample. Brightness (L) of the biscuits was measured using a ColorFlex colorimeter (Hunter Associates Laboratory, Reston, VA, USA). Six biscuit pieces, packaged in a way that was air-tight and impermeable to moisture, were held for a week, then given to expert panelists (n=6) for sensory evaluation, according to their order of baking. Each biscuit sample was scored on a Visual Analogue Scale, for which the extreme ends had numbers of 0 (the least desirable) and 10 (the most desirable) for various characteristics, and 5 (for reference product) as a benchmark score. Textural hardness, dispersion in the mouth after first bite, and after-taste

were scored, compared to reference commercial products, and at the end of the evaluation, the panelists were asked about preferences.

2.4. HMW-GS and wheat-rye translocation

A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method, modified by Koyuncu (2009), was used to analyze HMW-GS composition. After five wheat kernels, randomly selected from a spike of each genotype, were crushed in a mortar, the non-gluten proteins were removed by sequential treatment with 70% (v/v) ethanol and 50% (v/v) 1-propanol. The gluten proteins were then extracted and reduced, and the HMW-GS were analyzed using the SDS-PAGE system (Singh et al., 1991). DNA isolations were made from young leaves of the soft wheat lines, when plants were in a period of 3-4 tillers. The Sec1 gene marker was used to analyze for wheat-rye translocations (Yamamoto & Mukai, 2005).

2.5. Statistical analysis

Statistical analyses were carried out using the JMP 13.0.0 software (SAS Institute, Cary, NC, USA) statistics program package. Field experiments, involving eleven promising homozygous soft wheat lines, were established in a randomized complete-block design with four replications. The replications of samples, seven grown under rainfed and five under irrigated conditions within each crop year, were combined, and all laboratory tests were conducted with two replications. The physical and sensory analyses of the biscuit samples belonging to the soft- and hard-dough groups were evaluated separately. All data were evaluated using one-way analyses of variance, and means were compared using the Student's test ($P<0.05$).

3. Results and Discussion

3.1. Technological properties of the wheat genotypes and resulting flours of the hard- and soft-dough groups

The HMW-GS compositions, in the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, as determined from SDS-PAGE, and results for some technological quality properties of the wheat lines and their flours developed for soft- and hard-dough biscuit groups, are shown in Table 1. Results from the SDS-PAGE of the HMW-GS of the hard- and soft-dough groups of the biscuit wheats are shown in Figure 1. In this study, 4 of the 5 wheat genotypes in the soft-dough group carried the null allele in the *Glu-A1* locus, and the other genotype, line EK24, carried subunit 1. The seven wheat lines in the hard-dough group did not carry the null allele. Lines ES9 and ES11 had subunit 2*, and the others had subunit 1 in the *Glu-A1* locus. Interestingly, subunit 7x + 8y was only present in the soft-dough group lines EK7 and EK21. In contrast, the hard-dough group lines mostly carried subunit 7x + 9y in the *Glu-B1* locus. Only line ES18 in the soft-dough group carried subunit 7x + 9y. Subunit 7 was found in two lines in each group. None of the wheat lines carried subunit 17x + 18y encoded by *Glu-B1*. While all seven of the wheat lines in the hard-dough group carried subunit 5x + 10y, indicating high gluten strength in *Glu-D1*, 4 out of 5 of the wheat lines in the soft-dough group carried subunit 2x + 12y, associated with low gluten strength. Lines EK16 and ES7 in the hard-dough group and lines EK7 and ES18 in the soft-dough group had the 1B/1R wheat (*Triticum aestivum* L.)-rye (*Secale cereale* L.) translocation (as shown in Figure 2 and Table 1), which results in decreased gluten strength, mixing tolerance, and bread volume (Pena et al., 1990; Graybosch, 2001), because of the weakening effect of the low molecular weight gluten subunits of rye (Amiour et al., 2002).

When the technological properties of the eleven flours in the hard- and the soft-dough biscuit groups were compared, there were no significant differences in the wet and in the dry gluten contents of the samples. In this case, protein (gluten) quality, due to genotypic factors, revealed the differences among the samples. The three-year lactic acid SRC (LASRC) data for the EK4, EK7, EK16, EK21, and EK24 flours were evaluated in a previous study (Karaduman et al., 2020). The LASRC results for these flours in the present study, shown in Table 1, were from the first two years of that previous study.

In the present study, ZSed, gluten index, and LASRC values were used to evaluate the gluten strength of the flours in both biscuit wheat groups. In many soft wheat breeding programs, tests using lactic acid are often used for evaluations of the gluten strength of wheat genotypes (Guzman et al., 2016b; Karaduman, 2020). The SRC method was originally developed to measure the solvent-holding capacity of soft wheat flours (Slade & Levine, 1994), and has become widely used to evaluate the gluten strength of both soft and hard wheat flours (Kweon et al., 2011, 2014). In the present study, there were statistically significant differences among the eleven flour samples, in terms of their ZSed, LASRC, and gluten index values. As shown in Table 1, the averages of the ZSed and LASRC values of the flours from the wheat genotypes belonging to the hard- and soft-dough biscuit groups were 24.8 and 14.5 ml and 109.1 and 91.3%, respectively.

The gluten index values of the flours in the soft-dough biscuit group were quite low (<25.0 %), except for line EK21 (37.8%). The ZSed and gluten index values for the line EK21 flour were higher than those for the other flours of the soft-dough group. Also, the LASRC value for the EK21 flour was the highest in the soft-dough group (105.1%), even higher than that for the line ES7 flour and close to those for the line EK16 and ES1 flours (105.5 and 105.2%, respectively) in the hard-dough biscuit group. The higher gluten strength of line EK21 was attributable to its HMW-GS 5x + 10y. It was believed that this subunit 5x + 10y strengthened the gluten, despite the presence of the null allele in *Glu-A1*, which weakened the flour gluten in the other wheat lines of the soft-dough group. Thus, the presence of subunit 5x + 10y is not desirable in flours preferred for soft-dough biscuit products. Lines EK7 and ES18 in the soft-dough group carried the null allele in *Glu-A1* and subunit 2x + 12y in *Glu-D1*, as well as the 1B/1R wheat-rye translocation, resulting in quite low LASRC (<85.0%), ZSed (8.0 and 16.0 ml), and gluten index (<20.0%) values. Line EK7 flour also had the lowest water SRC (WSRC) value (51.4%) among the wheat lines of the soft-dough group, which is another desirable aspect of flours preferred for soft wheat biscuit products (Kweon et al., 2011, 2014). In comparison, although they did not have the 1B/1R wheat-rye translocation, the line EK24 and ES23 flours had low ZSed and gluten index values. Even though the ZSed value for line EK24 flour was very low, its LASRC value was medium-high (97.6%) within the soft-dough group. The remarkable situation for the two lines, EK24 and ES23, was that they carried subunit 7 in *Glu-B1* and subunit 2x + 12y in *Glu-D1*. For that reason, when low gluten strength is the aim in the soft-dough group of flours for such biscuit products, it could be advantageous to target those particular subunits, in the *Glu-B1* and *Glu-D1* loci, in soft wheat breeding programs.

Table 1. The HMW-GS compositions and results for some technological properties of the wheat genotypes and their flours in the hard- and soft-dough biscuit groups

Gen	Group	Hardness	Glu-A1	Glu-B1	Glu-D1	1B/1R	Wet Gluten Content%	Dry Gluten Content %	Gluten Index %	Zeleny Sedimentation Volume, ml	Water-SRC, %	Lactic acid-SRC, %	Sucrose-SRC, %	Sodium carbonate-SRC, %	Damaged Starch, %	Falling Number, s
ES1	Hard DG	medium	1	7+9	5+10	no	29.2±0.5	9.8±0.4	90.0±14.1	28.5±2.1	59.0±4.4	105.2±8.3	77.4±5.0	72.4±4.9	16.6±2.5	344±54
EK4	Hard DG	very soft	1	7+9	5+10	no	32.5±2.8	10.4±0.5	49.5±13.4	20.0±7.1	52.7±2.8	116.9±9.5	77.9±5.6	69.0±4.2	16.1±5.2	430±38
ES9	Hard DG	very soft	2*	7	5+10	no	30.0±1.7	10.4±0.9	87.5±17.7	29.0±1.4	55.6±4.3	116.8±10.1	80.9±6.7	73.1±7.7	14.0±2.1	287±2
ES11	Hard DG	very soft	2*	7	5+10	no	28.4±0.5	9.7±0.4	93.5±9.2	27.0±5.7	55.6±3.0	111.5±8.5	78.8±4.9	71.0±6.2	17.4±0.8	318±3
EK16	Hard DG	soft	1	7+9	5+10	yes	29.9±1.6	9.3±0.2	51.5±2.1	21.5±7.8	51.7±2.3	105.5±9.7	78.0±5.1	70.7±2.3	18.8±2.9	360±49
ES7	Hard DG	very soft	1	7+9	5+10	yes	28.8±2.4	9.2±0.4	75.0±24.0	23.0±2.8	55.2±3.2	98.7±5.4	81.8±5.2	75.7±0.0	13.7±1.1	255±25
		Mean-hard DG					**29.8**	**9.8**	**74.5**	**24.8**	**54.2**	**109.1**	**79.1**	**72.0**	**16.1**	**332**
EK7	Soft DG	soft	null	7+8	2+12	yes	30.8±7.5	10.0±2.2	19.5±27.6	8.0±0.0	51.4±2.8	82.9±8.9	74.5±4.6	69.2±4.1	17.6±6.6	399±37
ES18	Soft DG	very soft	null	7+9	2+12	yes	33.3±1.6	11.8±1.1	11.8±16.6	16.0±1.4	54.5±2.7	84.9±6.1	76.2±3.9	72.0±5.2	14.1±2.4	234±2
EK21	Soft DG	soft	null	7+8	5+10	no	29.1±1.8	9.4±0.6	37.8±5.3	18.0±4.2	52.2±3.0	105.1±9.1	75.2±4.6	67.7±4.0	18.1±1.0	382±27
ES23	Soft DG	soft	null	7	2+12	no	29.8±8.8	12.0±0.2	20.0±28.3	16.0±1.4	55.7±3.5	86.0±7.1	77.8±5.2	70.9±5.3	16.3±4.1	334±22
EK24	Soft DG	very soft	1	7	2+12	no	28.1±4.0	8.7±0.9	22.0±9.9	10.5±2.1	53.8±3.1	97.6±9.1	79.1±5.1	72.4±4.7	18.2±0.1	378±38
		Mean-soft DG					**30.2**	**10.3**	**22.2**	**14.5**	**53.5**	**91.3**	**76.6**	**70.4**	**16.8**	**346**
		CV (%)					13.6	9.2	7.1	14.6	4.5	8.6	5.2	5.9	17.2	9.7
		LSD					ns	ns	36.3**	6.4**	1.4**	5.2**	2.4**	2.5**	ns	73**

Data is presented as mean ± SD; *P <0.05; **P< 0.01; Gen: Genotype; SD: standard deviation of the means; ns: non-significant; DG: Dough group; 1B/1R: wheat-rye translocation; WGlu: Wet gluten content; DGlu: Dry gluten content; GI: Gluten index value; ZSed: Zeleny sedimentation volume; WSRC: Water solvent retention capacity; LASRC: Lactic acid solvent retention capacity; SUSRC: Sucrose solvent retention capacity; SCARBSRC: Sodium carbonate solvent retention capacity; DS: Damaged starch content; FN: Hagberg falling number value. [It should be noted that the SUCSRC data shown here were determined using a non-standard sucrose solution made from 50 g sucrose + 100 g water, which deviated from the standard 50 w% sucrose solution (made from 50 g sucrose + 50 g water) of AACC Method 56-11 (AACC, 2010).]

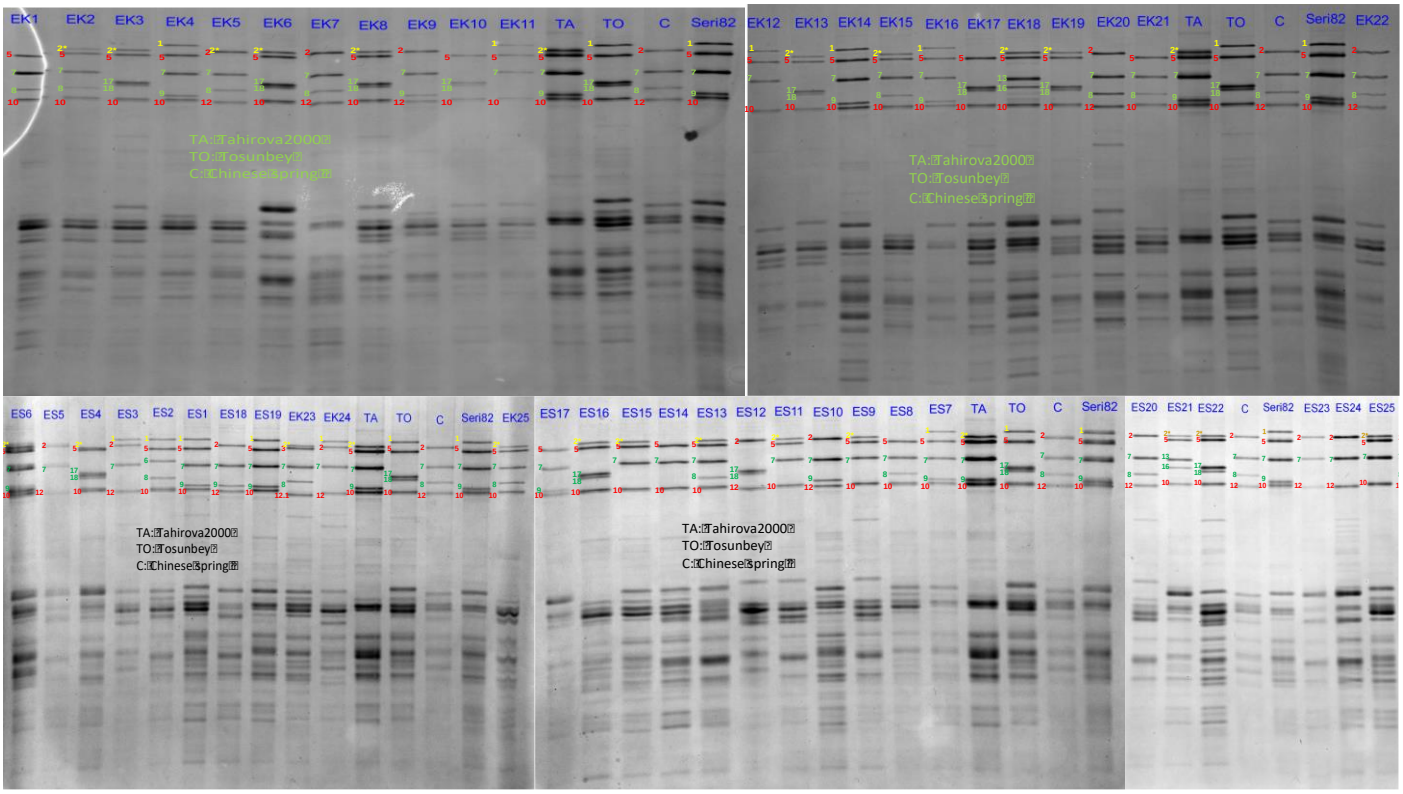

Figure 1. SDS-PAGE of the HMW-GS of the hard- and soft-dough groups of biscuit wheats

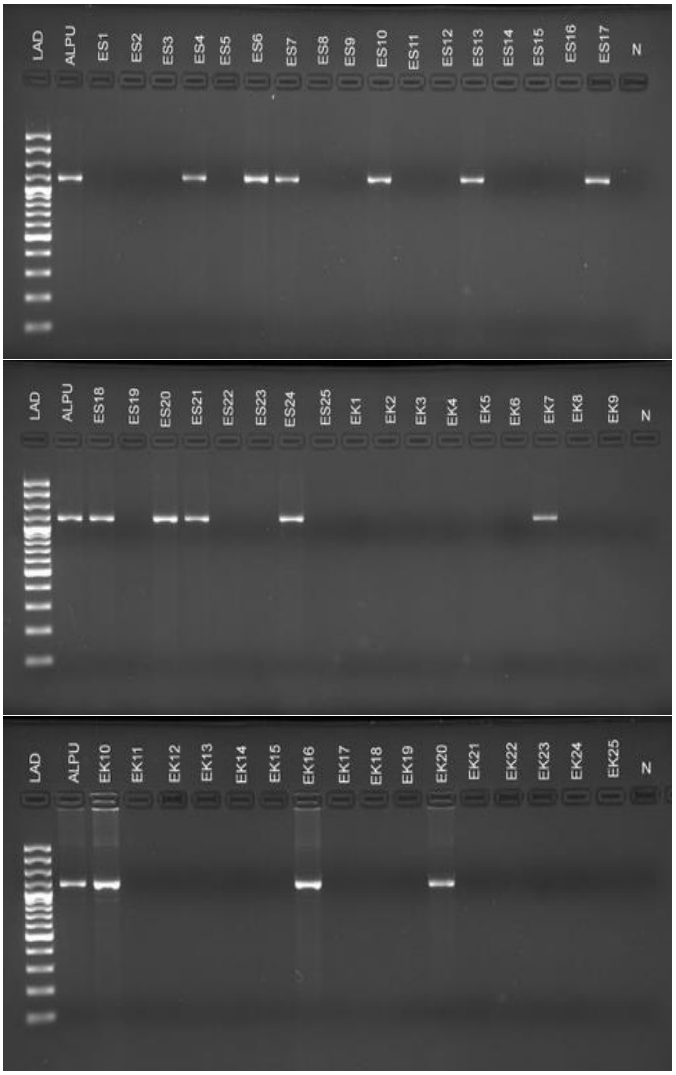

Figure 2. The 1B/1R wheat-rye translocations of the wheat genotypes

Generally, the ZSed, LASRC, and gluten index values for the flours of wheat lines ES9 and ES11, belonging to the hard-dough group and carrying subunit 2* in *Glu-A1*, were high.

In the present study, this high gluten strength was thus attributable to subunit 2*. Therefore, the presence of this subunit could be used as a marker, in order to eliminate those wheat lines having too-high gluten strength in certain soft wheat breeding programs. In comparison, with medium-level LASRC, ZSed, and gluten index values, the flours of wheat lines EK16 and ES7, which carried the 1B/1R wheat-rye translocation and combinations of the HMW-GS 1, 7x + 9y, and 5x + 10y, were considered to have adequate gluten strength and extensibility for the hard-dough group of biscuit products. However, despite wheat lines EK4 and ES1 having similar HMW-GS compositions, and even without the rye gene translocation, the unacceptably high variability of the LASRC, ZSed, and gluten index values of their flours suggested that not only the HMW-GS glutenins, but also the low molecular weight glutenin subunits (LMW-GS), which constitute 75% of the total gluten, might have a significant effect on dough viscoelastic properties (Rasheed et al., 2015).

There were no significant differences between the hard- and soft-dough groups of biscuit flours, in terms of their amounts of damaged starch. The average amounts of damaged starch in all eleven flours varied from 13.7% (i.e. low) to 18.8% (i.e. medium-low). Among the six wheat lines with very soft endosperm, ES9, ES7, and ES18 flours had damaged starch levels of around 14.0%. In comparison, in the other three lines with very soft endosperm (EK4, ES11 and EK24), the damaged starch levels in the flours were somewhat higher. The amount of damaged starch in a given wheat flour can have a significant effect on gluten hydration and water absorption in a dough (Slade & Levine, 1994; Kweon et al., 2011, 2014; Finney & Bains, 2002). Thus, in our view, low-to-medium levels of damaged starch in biscuit flours for hard-dough products can have a positive effect on finished-product properties.

Hagberg falling number (FN) values are known to correlate

with alpha-amylase activity levels in wheats. As a result of low amylase activity, higher FN values are seen. It is considered preferable to have FN values above the 300.0±50.0 s level in flours for soft wheat-based products, because too-high amylase activity, resulting in too-soft and -sticky doughs, leads to an increased cookie spread ratio, thus causing a deleterious effect on the size of baked biscuits (Souza & Kweon, 2010). In the present study, although wheat lines ES18 and ES7 had somewhat higher amylase activity levels, and consequently showed lower FN values, those FN values were acceptable and did not negatively affect dough and product properties.

3.2. Sensory properties of the hard- and soft-dough biscuit products

Results for the moisture contents, physical and sensory properties of the soft-dough and hard-dough biscuit products produced from the eleven sample flours are shown in Table 2. The biscuit product moisture contents varied from 1.97 to 2.19% in hard-dough, and 2.55 and 2.72 % in soft-dough biscuits products. Photographs of the biscuit samples from the soft- (baby-type) and hard-dough (petit beurre) groups of wheat genotypes are shown in Figure 3. The petit beurre biscuit is a wire-cut-type product, for which the extensible, hard dough is thinned to a certain thickness and then cut with molds. The total formula level of water (i.e. water + liquid milk) is higher than that of fat, and some gluten network formation is desirable, in order to obtain the extensible dough required for processing this type of biscuit. In contrast, the baby-type biscuit is an extruded-type product that dissolves rapidly in the mouth. A creamy dough, which is not extensible and is formulated with much more fat than water, is used in the production of this type of biscuit. Too-high gluten strength causes a too-hard, non-homogeneous surface and inappropriate biscuit size in baby-type biscuits (Karaduman, 2020). In the present study, the physical and sensory properties of these two soft- and hard-dough biscuit products were evaluated against the corresponding properties of their respective reference biscuit samples (shown in Table 2). No statistically significant differences were found for the width and length values of the baby-type biscuit samples made from the soft-dough group of wheat lines. There were statistically significant differences only for the thickness and brightness values of the soft-dough group products. The thickness of the baby-type biscuits made from line EK7 flour was quite small, compared to that of the reference. This EK7 line had the null and 2x + 12y subunits, and also the 1B/1R wheat-rye translocation. Baby-type biscuit samples made with the flour from this wheat line were also significantly lighter in color brightness (L = 76.4) than biscuit samples made with flours from the the other soft-dough group lines. The sensory scores of the line EK7 baby-type biscuits, for “taste after eating” and “like preference”, were lower than those for the reference product and the other soft-dough group samples. The WSRC, LASRC, and SUSRC values for this line EK7 flour were also lower than those for all the other wheat line flours. The dough made from this line EK7 flour was of inferior quality, with very weak dough properties, whereas at least slightly better gluten quality is required of flours used for such baby-typebiscuits. The presence of the rye gene translocation, along with HMW-GS combinations by which gluten strength is already weakened, will create much weaker gluten, and can thus result in unprocessable doughs and negative product properties. Therefore, it is necessary to be cautious in searching for rye gene translocations in selections of particular genotypes in wheat breeding programs.

In comparison to EK7, although line ES18 wheat had similar HMW-GS, other than the allele in *Glu-B1*, and the 1B/1R wheat-rye translocation, the resulting baby-type biscuit hardness and texture properties were closer to those of the reference sample, and therefore much preferred in sensory evaluations. Notably, the “disperse in the mouth” and “like preference” scores for line ES18 biscuits were higher than those for the reference sample. Thus, it was believed that the 7x + 9y subunit in *Glu-B1* of wheat line ES18 was considered to result in suitable flour for the production of baby-type biscuits, by increasing somewhat the flour's gluten strength, compared to that of line EK7. In addition to ES18 flour's gluten strength properties, its WSRC, SCARBSRC, and SUCSRC values were also higher than those for EK7 flour. Those results suggested that the gluten-weakening effect of the rye gene translocation was balanced by somewhat higher gluten quality, as well as by those properties represented by the higher SRC values (Kweon et al., 2011). A determination of the LMW-GS of these two wheat genotypes would allow for a more comprehensive assessment in this respect. The dough properties obtained with line ES18 flour were very suitable for the extrusion processing of baby-type biscuits. Although the flour obtained from wheat line ES18 had higher alpha-amylase activity, and consequently a lower FN value than those for the other soft-dough group flours, that characteristic did not manifest a negative effect on biscuit product properties.

Wheat line EK21, which carried subunit 5x + 10y in *Glu-D1*, had relatively high gluten strength (LASRC = 105.1%), while its other flour SRC values were similar to those for the flours of the other wheat lines in the soft-dough group. In this case, while EK21's biscuit length and width were similar to those of the reference sample, its thickness was increased considerably, as expected (Kweon et al., 2011, 2014). This increase in biscuit thickness led to a lowered hardness value for the product, and this flour's too-high LASRC value resulted in a too-strong and thus unprocessable baby-type biscuit dough. Too-high gluten strength appeared to negatively affect all the sensory evaluation properties of the biscuits made from EK21 flour.

Wheat lines ES23 and EK24 both had subunits 7 and 2 + 12 in *Glu-B1* and *Glu-D1*, respectively, but carried different alleles, null and 1, respectively, in *Glu-A1*. In general, line ES23 flour produced doughs with suitable viscoelastic properties, and baby-type biscuits with physical and sensory properties similar to those of the reference sample. For line EK24 flour, its biscuit physical properties were similar to those of the reference sample, and its biscuit sensory scores were above those of the reference sample for “taste after eating” and “like preference”. However, the line EK24 biscuits had a too-high hardness value (texture meter) and too-low sensory scores for “texture” and “disperse in mouth”, even though the EK24 flour doughs' viscoelastic properties were judged to be suitable. Although the dry gluten content of the line EK24 flour was relatively low, its damaged starch content was relatively high, which could have led to over-hydration of the dough and caused some gluten network formation. Furthermore, it was suggested that the presence of allele 1 led to increased gluten strength, as indicated by the relatively high LASRC value for the EK24 flour, and, consequently, impaired the desired sensory properties of the extruded soft-dough product (Kweon et al., 2014). Therefore, when allele 1 is found in *Glu-A1*, one should be cautious about selecting such wheat genotypes for production of soft-dough biscuit products.

There were no statistically significant differences for any of the physical property values for the petit beurre biscuit samples prepared from the flours of the six wheat lines in the hard-dough group. Among those wheat lines, ES9 and ES11

Table 2. Results for the moisture contents and physical and sensory properties of the soft-dough (baby-type) and hard-dough (petit beurre) biscuit products produced from the flours of the eleven wheat genotypes

		Physical Properties							Sensory Evaluation			
Gen	**Group**	**Moisture Content,%**	**Width mm**	**Length mm**	**Thickness mm**	**Brightness L**	**Hardness g/f**	**Texture**	**Disperse in mouth**	**Taste**	**Like preference**	**User evaluation**
ES1	Hard DG	2.04±0.11	51.2±.6	64.9±0.1	5.9±0.5	73.2±0.5	1316±108	4.9±0.0	5.2±0.1	5.0±0.0	5.1±0.0	Strong dough, easy kneading
EK4	Hard DG	1.97±0.06	51.1±4.4	68.8±5.5	5.9±0.3	70.1±4.4	1339±215	5.1±0.4	5.0±0.2	4.1±0.9	4.7±0.9	Strong dough, rapid color formation
ES9	Hard DG	2.02±0.08	51.4±5.3	70.6±5.3	5.7±0.7	73.2±7.1	1716±41	3.9±0.3	3.5±0.2	4.4±0.6	3.9±0.1	Strong dough, lumpy
ES11	Hard DG	2.19±0.08	50.5±2.1	67.9±6.1	7.1±0.2	64.0±8.7	1767±810	3.6±1.0	4.3±0.5	4.1±0.2	4.1±0.1	Strong dough
EK16	Hard DG	2.03±0.17	53.7±0.7	65.2±0.3	5.9±0.0	73.0±0.1	1352±32	5.0±0.1	5.2±0.1	5.0±0.1	5.1±0.1	Suitable dough
ES7	Hard DG	2.04±0.07	54.2±0.2	65.2±0.3	6.0±0.1	73.6±0.8	1238±79	5.1±0.2	5.4±0.1	4.9±0.1	5.2±0.0	Suitable dough
Ref	Hard DG	2.06	54.3	65.2	6.0	73.6	1221	5.0	5.0	5.0	5.0	
Mean		**2.05**	**52.0**	**67.1**	**6.1**	**71.2**	**1455**	**4.6**	**4.8**	**4.6**	**4.7**	
CV (%)		5.4	4.2	4.6	6.7	5.4	23.0	10.1	5.0	10.0	7.5	
LSD		ns	ns	ns	ns	ns	ns	1.19*	0.62**	ns	0.90*	
EK7	Soft DG	2.72±0.08	28.5±1.0	48.6±1.0	8.8±0.3	76.4±2.3	1564±296	4.7±1.0	4.9±0.7	4.0±1.2	4.1±0.1	Weak dough, difficult shape
ES18	Soft DG	2.64±0.13	28.0±0.1	47.1±1.2	10.2±0.1	73.7±.0	1632±78	5.0±0.1	5.2±0.1	4.9±0.0	5.2±0.1	Very suitable dough
EK21	Soft DG	2.55±0.02	27.4±3.2	45.2±2.7	12.3±0.7	75.7±0.9	1302±388	4.3±1.2	4.2±0.6	4.8±0.7	4.3±0.2	Strong dough, difficult shape, late color formation
ES23	Soft DG	2.71±0.02	28.1±0.4	46.3±0.2	10.8±0.2	74.4±0.7	1530±74	5.1±0.2	5.0±0.2	4.9±0.0	5.1±0.2	Suitable dough, easy to shape
EK24	Soft DG	2.63±0.08	27.9±0.5	47.1±1.1	11.1±0.6	72.5±0.6	1904±366	4.3±1.2	4.0±1.4	5.1±0.3	5.2±0.2	Suitable dough, easy to shape
Ref	Soft DG	2.68	27.4	45.9	10.5	73.1	1595	5.0	5.0	5.0	5.0	
Mean		**2.65**	**28.0**	**46.9**	**10.6**	**74.5**	**1586**	**4.7**	**4.7**	**4.7**	**4.7**	
CV (%)		3.3	5.5	2.0	4.5	1.1	17.5	21.3	18.6	14.6	3.4	
LSD		ns	ns	ns	1.33*	2.29*	ns	ns	ns	ns	0.44**	

Data is presented as mean ± SD; *P<0.05; **P< 0.01; Gen: Genotype; Ref: reference biscuit sample; DG: Dough group; SD: standard deviation of the means; ns: non-significant

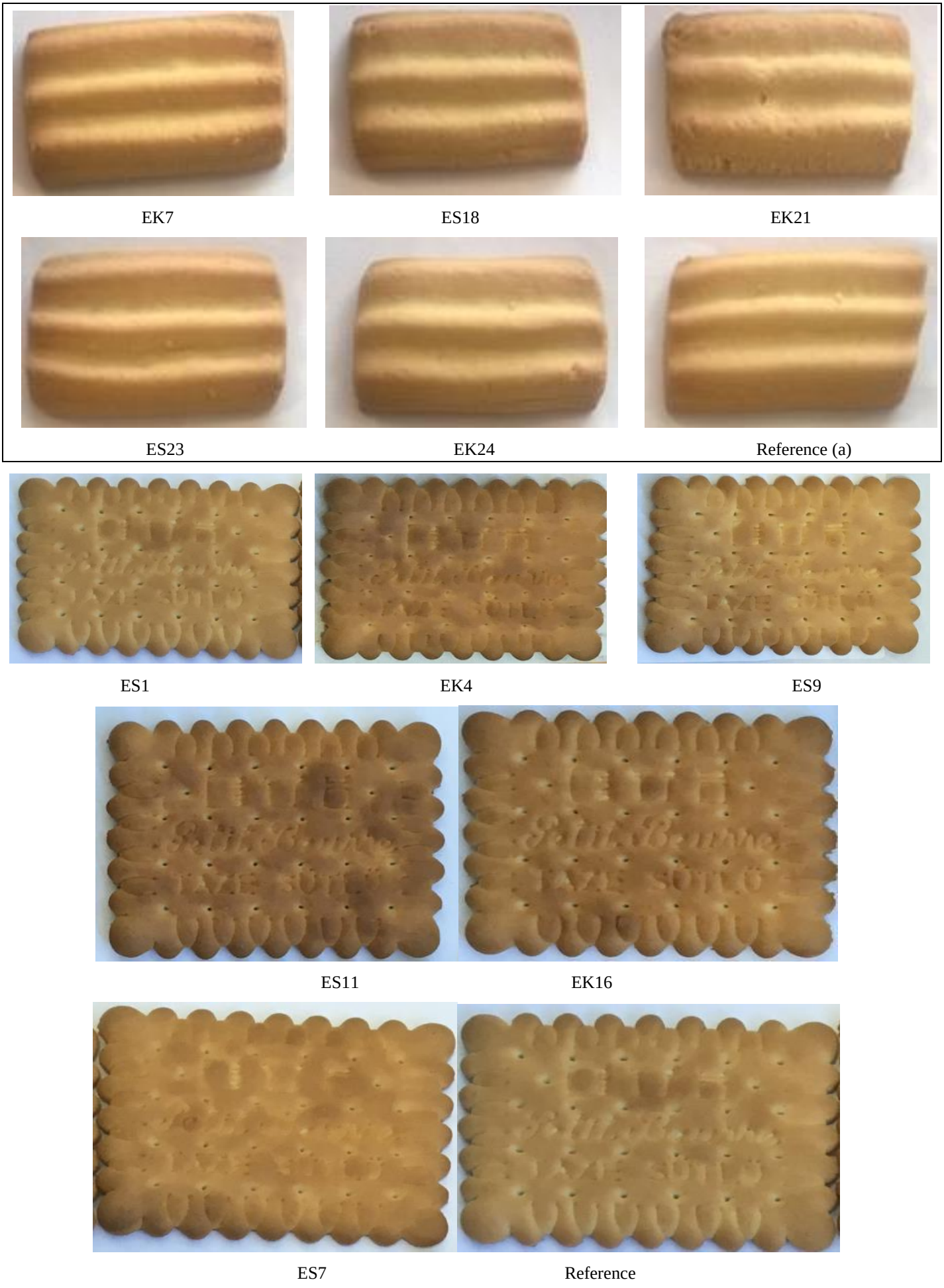

Figure 3. a) Biscuits samples (baby-type) from the soft-dough group of wheat genotypes, and b) biscuit samples (petit beurre) from the hard-dough group of wheat genotype

carried subunits 2* in *Glu-A1* and 7 in *Glu-B1*, unlike the other four lines. In those two lines, although subunit 7 had a gluten-weakening effect, subunit 2* strengthened the gluten in the flours, so their ZSed, LASRC, and gluten index values were increased. Consequently, the widths of the resulting biscuits were relatively lower and their lengths were relatively longer, compared to the reference sample. The thickness value of the line ES11 biscuits and the texture meter hardness values for both the ES9 and ES11 biscuits were much higher than the corresponding values for the reference sample. Moreover, the color brightness value of the line ES11 biscuits was much lower (i.e. darker surface, as can be clearly seen from the photos in Figure 3) than that for the reference. Too-strong and unprocessable doughs were obtained with the flours from these two wheat lines, and all the sensory scores for their respective biscuits were quite low. Even though relatively low starch damage was found in the line ES9 flour, its biscuit product properties were found to be undesirable. This result suggested that subunit 2* was the undesirable culprit, especially for such hard-dough biscuit production. If subunit 2* and the rye gene translocation were both present, it might be necessary to compensate by adjusting the dough and product properties.

The other four wheat lines in the hard-dough group carried subunits 1 in *Glu-A1*, 7x + 9y in *Glu-B1*, and 5x + 10y in *Glu-A1*. Lines EK16 and ES7 also carried the 1B/1R wheat-rye translocation. Compared to the reference sample, biscuits made from the flours of the latter two wheat lines had similar or better scores in their physical and dough properties and sensory evaluations. In particular, texture meter hardness, “disperse in mouth”, and “like preference” scores for the line ES7 product were especially good. These results suggested that the effect of increasing gluten strength, due to the 5x + 10y subunit, was desirably reduced by the presence of the rye gene translocation, even though the WSRC, SUCSRC, and SCARBSRC values for the ES7 flour and the damaged starch content in the EK16 flour were all relatively high. Excellent viscoelastic properties were obtained for the hard-dough biscuit doughs made from the flours carrying subunits 1 in *Glu-A1* and 7x + 9y in *Glu-B1*, along with the 1B/1R wheat-rye translocation. [It should be mentioned that, while evaluating this subject matter, definition of the existing LMW-GS composition might provide further clarity.] Flours from the other two wheat lines in the hard-dough group, ES1 and EK4, produced biscuits with smaller widths than for the reference sample, and strong dough properties. The lengths of the line EK4 biscuits were also longer than that of the reference. In particular, the “taste after eating” and "like preference" scores for the line EK4 biscuits were lower than those for the reference sample, while the line ES1 biscuits scored well in all their sensory characteristics. Even though the ZSed, gluten index, and WSRC values for line ES1 flour were much higher than those for line EK4 flour, the former's LASRC value was lower, and the petit beurre biscuits produced from the ES1 flour were well-liked by the sensory panelists. This finding suggested that, in the hard-dough group, for which some flour gluten formation and dough extensibility are desired, defining the specific LMW-GS compositions in the wheat genotypes could be even more critical than for the soft-dough group of wheats. Transferring rye gene translocations to wheat lines such as EK4 and ES1 might enable the production of doughs having suitable viscoelastic properties and resulting biscuits having suitable sizes.

4. Conclusions

This study demonstrated that the 5x + 10y HMW-G subunit in certain soft wheat genotypes led to flours having increased gluten strength, which negatively affected the physical and sensory properties of the resulting soft-dough biscuit products. Especially when allele 1 is found in *Glu-A1*, it is essential that alleles other than 5x + 10y be carried in the *Glu-B1 and Glu-D1* loci, in order to avoid overly increased gluten strength in such flours for soft-dough products. For example, HMW-G subunit 7 in *Glu-B1*, together with subunit 2x + 12y in *Glu-D1*, should be targeted in soft wheat breeding programs aimed at soft-dough biscuit products. Because of the large gluten-weakening effect of the 1B/1R wheat-rye translocation, one should be cautious whenever such rye gene translocations are found in soft wheatlines to be selected for soft-dough biscuit products. For wheat genotypes and suitable flours for hard-dough biscuit products, subunit 2* can be used as a marker, in order to eliminate those wheat lines in breeding programs, because of consequent too-high dough and gluten strength. Combinations of the HMW-G subunits 1, 7x + 9y, and 5x + 10y, together with the 1B/1R wheat-rye translocation, were determined to yield sufficiently high gluten strength and enough dough extensibility for production of this type of hard-dough group of products, exemplified by by petit beurre biscuits. Moreover, for wheat genotypes and suitable flours for the hard-dough group of products, defining the composition of the LMW-GS, along with the HMW-GS, might be even more critical than for the case of soft-dough products. Transferring rye gene translocations to selected wheat genotypes might result in favorable dough and biscuit product properties for those wheat lines already carrying subunit 5x + 10y.

Ethical responsibility

All procedures performed in studies involving human participants were as per the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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