

Effect of ethanolic extract of *Cephalaria syriaca* on dough rheological properties from different wheat flour blends: A comparative study with ascorbic acid

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ABSTRACT

Cephalaria syriaca is an annual plant that is widely grown in wheat fields and is frequently mixed with wheat crops during harvesting. In this study, contribution of incorporation of ethanolic extract of *C. syriaca* (CSE, 0.3% w/w) on viscoelastic properties of wheat flours with different protein contents and qualities was investigated by farinograph and extensograph studies in comparison with the effect of ascorbic acid (AA). In general, both CSE and AA improved rheological characteristics of the flours in correlation with their protein contents and quality. Water absorption of the hard flour (HF) was slightly increased (to 61.1% from 60.5%) by CSE addition while AA did not make it any contribution. Energy levels of the doughs containing CSE varied from 96 cm² to 118 cm² which were significantly higher than those of the AA supplemented samples. Ratio numbers varying from 3.3 to 6.1, which were lower and higher than the control and AA supplemented samples, respectively were obtained at the samples containing CSE at all proving times (49, 90 and 135 min). In conclusion, this study confirmed that CSE could be combined with wheat flour in order to develop its viscoelastic properties as a natural dough improver.

1. Introduction

Traditionally, most of bread varieties are manufactured using wheat flour. Quality of bread during its shelf life is widely dependent to flour quality and addition of other ingredients. In commercial breadmaking in many countries as well as in Turkey, wheat flour, salt, water and yeast (*Saccharomyces cerevisiae*) are the main constituents of bread (Goesaert et al., 2005). The main function of starch that constitutes 70-75 % of wheat flour is water absorption (~45 %) while no additional role of starch in bread is clearly known. However, wheat gluten is the main compound affecting bread quality, both quantity and composition of gluten is important (Southan & MacRitchie, 1999). In dough kneading step, gluten structure forms a continuous protein network that gives the viscoelasticity of flour dough (Singh & MacRitchie, 2001).

Since weak wheat flours does not contain sufficient amount of gluten to form the protein network during kneading and yeast fermentation, several additives are used in order to improve the dough quality. Ascorbic acid is the most common compound used for dough improver, dough strengthening effect of which

was firstly established by Jorgensen (1935) as can be measured by farinograph and extensograph studies. Although mechanism of action of ascorbic acid is not exactly known, several theories has been presented. In the case of the most known theory, ascorbic acid forms dehydroascorbic acid as a result of oxidation by ascorbate oxidase, followed by formation of disulfide bonds by oxidation of endogenous glutathione (Koehler, 2003). Several chemical dough improvers such as sodium alginate, xanthan gum, hydroxypropylmethylcellulose, DATEM and sucrose esters have also been tested for their dough improvement effect. However, they have not been employed as much as ascorbic acid in the industry (Barrett et al., 2002; Guarda et al., 2004; Ribotta et al., 2005; Ribotta et al., 2004; Rosell, et al., 2001a; Rosell et al., 2001b).

Cephalaria syriaca is an annual common weed that is widely associated with wheat fields. Its main growth area is Eastern Mediterranean regions (Musselman, 2000). In spite of differences between growth forms of *C. syriaca* and wheat, *Cephalaria* seeds are very similar to wheat grains (Boz & Karaoglu, 2013). Strengthening effect of *C. syriaca* fractions (esp. flour) on the properties of wheat flours has been demonstrated by several studies (Karaoglu, 2006; Karaoglu,

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2012). *C. syriaca* is also traditionally used for increasing the strength of weak flours while its addition is also known to improve bread properties (Altiniğne & Saygin, 1985; Boz & Karaoglu, 2013). However, the most critical drawback of *C. syriaca* seeds is the bitterness that is resulted from the presence of glycosides consisting of non-toxic sugar esters (Karaoglu, 2012). Therefore, addition of *C. syriaca* flour in higher amounts into wheat flour gives the baked product a bitter taste as well as dark color. In this study, we aimed to minimize the color and bitterness drawbacks of *C. syriaca* by extracting functional compounds from the seed using ethanol and to test the efficiency of ethanolic *C. syriaca* extract on wheat dough properties comparatively with ascorbic acid.

2. Materials and Methods

2.1. Materials

Defatted *Cephalaria syriaca* powder was supplied by Ziya Organic Farming Enterprises in 2016. Soft and hard wheat flours were provided from Istanbul Halk Ekmek, Turkey. L-ascorbic acid was supplied by Vankim Ltd. (Istanbul, Turkey). Ethanol that was used for extraction was analytical grade and purchased from Merck (Germany). All other chemicals used were analytical or technical grade. Soft and hard flours were used in farinograph and extensograph studies after mixing at different ratios as seed in Table 1.

2.2. Production of *Cephalaria* extracts

Ethanolic extract of defatted *Cephalaria* (CEE) powder was produced using Soxhlet extraction system followed by a rotary evaporation step for removing of ethanol. Firstly, 50 g of powder was treated with ethanol for 6 h using a Soxhlet apparatus. Then the extract was concentrated at reduced pressure and temperature using a rotary evaporator (R-100, Buchi Labortechnik AG, Switzerland). Extraction yield was determined as 20% for defatted *C. syriaca* powder.

2.3. Determination of quality properties of wheat flour mixtures

In this study, for obtaining of wheat flour mixtures with different quality properties, hard and soft wheat flours were mixed at different ratios (0:1, 2.5:7.5, 1:1, 7.5:2.5 and 1:0) and coded with different IDs as seed in Table 1. Wet gluten, dry gluten, gluten index, normal sedimentation, delayed sedimentation, moisture, ash and protein contents of the flour mixes were determined based on the approved AACC method (AACC, 1999a).

2.4. Farinograph studies

Prior to experiments, wheat flour samples were supplemented with *C. syriaca* ethanolic extract (CEE, 0.3%

w/w). Ascorbic acid (AA) was also supplemented at the same ratio and its effect was compared with that of the extract.

Behavior of wheat flours supplemented with the extract or ascorbic acid during mixing was determined using a farinograph (Farinograph®-AT, Brabender, Duisburg, Germany) integrated with water dosing based on the method described by (AACC, 1999b) with the Method 54-22.01. Water absorption (WA, the amount (%)) of water to obtain dough consistency of 500 Brabender units (BU), stability (time (min) during which the dough will keep its initial consistency) and development time (time (min) required to form the dough) parameters were obtained with the farinograms.

2.5. Extensograph studies

Extensograph behaviors of flour samples with different qualities supplemented with CEE or AA were determined according to the method described by AACC (1999b) with the Method 54-22.01. Energy (cm^2), resistance to extension (BU), extensibility (mm), maximum resistance (BU), ratio number and maximum ratio number were obtained with the extensograms.

2.6. Statistical analysis

In this study, the analyses were carried out in triplicate. Statistical analysis (analysis of variance, ANOVA) was performed using a Windows based statistical analysis software (SAS 8.2, SAS Institute, Cary, North Carolina, USA). The significance of the differences between the mean data was assessed using Duncan's multiple comparison test at a significance level of 95%.

3. Results and Discussion

3.1. Quality of flour blends

Quality characteristics of flour blends (Table 1) were analyzed prior to farinograph and extensograph studies. Ash, protein, moisture, sedimentation (normal and delayed), wet and dry gluten and gluten index values that are important quality parameters especially in baking procedures were evaluated. Analyzed quality parameters of flours are presented in the Table 2.

Gluten proteins in bread flour are major components of the dough growth and elasticity (Egesel et al., 2009; Schofield, 1994). In addition, it is also well known that there is a significant relationship between the gluten quality and the content and strength of wheat flour (Jirsa & Hruskova, 2005). The protein content of wheat flour consists mainly of gliadin and gluten in the gluten structure.

Gluten quality is the main criterion for evaluating the cooking characteristics of wheat flour especially in bread making.

Table1. Composition of hard wheat flour and soft wheat flour blends

Blend ID	Blend type	Hard wheat flour (g/100 g)	Soft wheat flour (g/100 g)
SF	Soft wheat flour	0	100
B1	Hard wheat flour+soft wheat flour	25	75
B2	Hard wheat flour+soft wheat flour	50	50
B3	Hard wheat flour+soft wheat flour	75	25
HF	Hard wheat flour	100	0

Table 2. Quality properties of flour blends

Sample	Wet gluten (g/100 g)	Dry gluten (g/100 g)	Gluten index (%)	NS	ZS	Moisture (g/100 g)	Ash (g/100 g)	Protein (g/ 100 g)
SF	21.47±0.03 ^d	6.25±0.22 ^d	99.00±1.00 ^a	30.50±0.50 ^d	35.50±0.50 ^b	13.60±0.01 ^b	0.53±0.01 ^e	10.24±0.00 ^e
B1	22.91±0.76 ^c	7.21±0.17 ^c	96.50±0.50 ^b	33.00±0.00 ^c	34.50±0.50 ^c	13.71±0.02 ^a	0.74±0.00 ^b	10.79±0.00 ^d
B2	24.46±0.37 ^b	7.99±0.23 ^b	92.50±0.50 ^d	33.00±0.00 ^c	36.00±0.00 ^b	13.50±0.00 ^c	0.66±0.00 ^d	11.11±0.04 ^c
B3	25.48±0.41 ^b	8.13±0.29 ^b	96.00±1.00 ^{bc}	35.50±0.50 ^b	38.00±0.00 ^a	13.50±0.00 ^c	0.72±0.00 ^c	11.90±0.01 ^b
HF	26.91±0.64 ^a	9.25±0.05 ^a	94.50±0.50 ^c	37.50±0.50 ^a	38.00±0.00 ^a	12.87±0.02 ^d	0.78±0.00 ^a	12.44±0.01 ^a

NS: Normal sedimentation; ZS: Zeleny sedimentation. a-e: The different lowercases within the same column show that the results are significantly different ($P < 0.05$).

Gluten index, which expresses the gluten quality as much as the amount of gluten that make up the skeleton structure of the dough, comes out as another important parameter in breads (Aydoğan et al., 2012). In this study, gluten index values of the flour samples were between 92.50% and 99.00%. For breadmaking, a flour is preferred to have a gluten index higher than 60% (Elgun et al., 2001). Wet gluten value also gives information about water binding capacity in the baking flour while both wet and dry gluten is indicators of flour quality. In terms of wet gluten, the wheat flour samples had levels varying from 21.47% to 26.91% and the values were in correlation with the relative levels of the soft and hard flours (Table 2). The results were in agreeable with the findings of Kulkarni et al. (1987) and Torbica et al. (2007).

Zeleny sedimentation is another test that gives information about the gluten quantity and quality of wheat flour. Higher zeleny sedimentation value indicates better quality of flour while bread made from the corresponding flour will be more voluminous (Elgun et al., 2001). According to TS 2004 wheat standard, zeleny sedimentation values higher than 36 mL, between 25 mL and 36 mL, between 16 mL and 24 mL and lower than 15 mL correspond to excellent, good, poor and very poor flour qualities (Elgun et al., 2001). In this study, Zeleny sedimentation values of the flour blends were all between 34.5 mL and 38 mL (Table 2), which indicates that the all flour samples had good and excellent or good qualities in terms of their Zeleny sedimentation.

The moisture and dry matter contents are critical parameters involved in the storage and processing of cereals as well as their pricing. As seen in Table 2, the flour samples had moisture levels ranging from 12.87% to 13.71%, which were in accordance with the Turkish Food Codex (2013) that specifies moisture levels for wheat flour to be under the maximum limit of 14.50%. The ash content of flours is an accepted criterion in almost every country with the determination of the yield of a flour or the yield of % flour and is used to classify flour. It is also used as a parameter for flour classification (Elgun et al., 2001). In general, lower ash level is desired in order to produce whiter products. In this study, the (plain) soft flour sample had the lowest ash content (0.53 %) while incorporation of hard flour caused higher ash contents. Except for the plain soft flour, the samples had ash levels in accordance with Turkish Food Codex (2013).

3.2. Farinograph

Various additives and processing aids are widely used today in order to eliminate some defects and to improve bread quality caused by the flour composition in breadmaking industry. Ascorbic acid has an important place in these bread additives and is widely used especially to improve bread quality of weak flours. Farinograms have been used in food industry to determine the dough mixing properties of flours used in breadmaking. Farinograph characteristics (water absorption, stability and development time) of the flour blends supplemented with CEE or AA are exhibited in Figure 1.

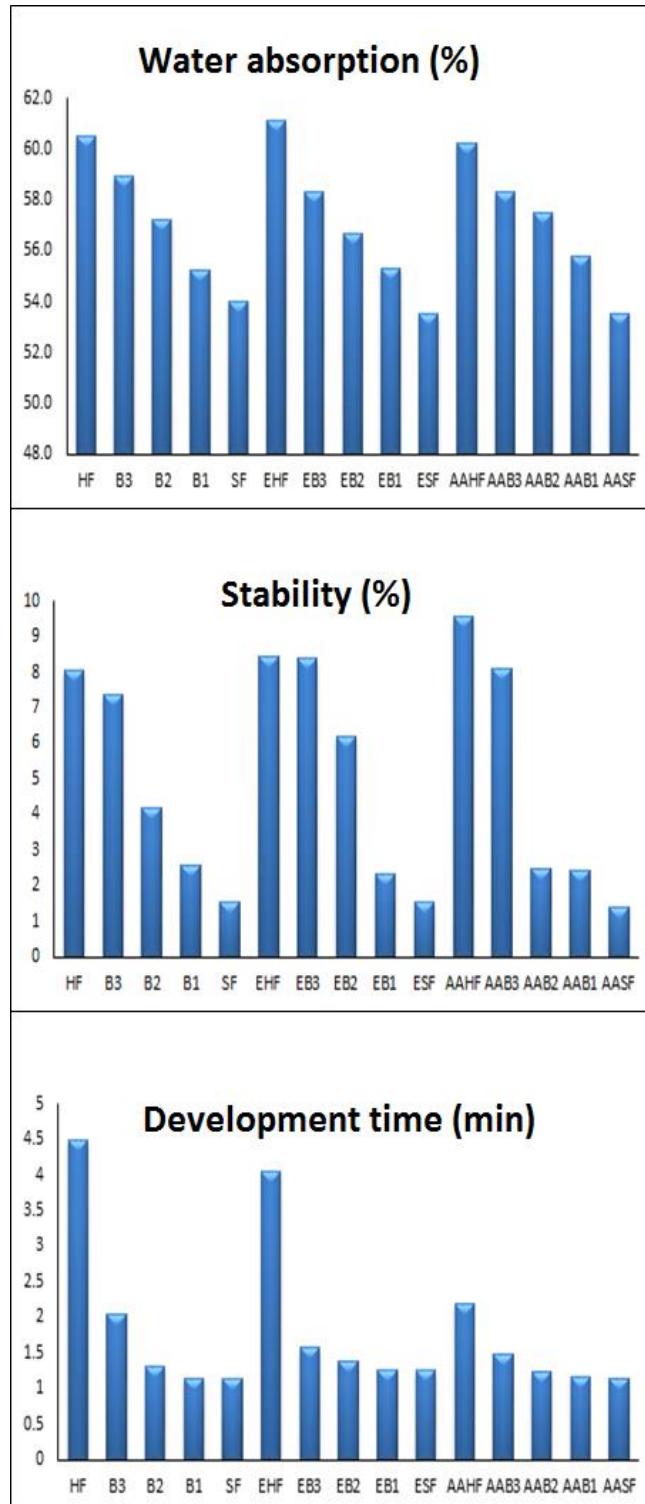


Figure 1. Farinograph properties of the flour blends supplemented with *Cephalaria syriaca* extract (CSE) in comparison with ascorbic acid (AA) supplementation (EHF: The hard flour sample supplemented with CSE, EB1: The flour sample B1 supplemented with CSE, EB2: The flour sample B2 supplemented with CSE, EB3: The flour sample B3 supplemented with CSE, ESF:

The soft flour sample supplemented with CSE, AAHF: The hard flour sample supplemented with AA, AAB1: The flour sample B1 supplemented with AA, AAB2: The flour sample B2 supplemented with AA, AAB3: The flour sample B3 supplemented with AA, AASF: The soft flour sample supplemented with AA)

As expected, the incorporation of CEE or AA into wheat flour caused significant changes in dough mixing behaviors. Water absorption levels of the flour samples containing CEE or AA varied from 53.5% to 60.5%. CEE incorporation provided the highest water absorption in the dough produced from hard flour (EHF) while AA did not do contribution to any of the flour samples. It was clear that water absorption levels of the dough samples were correlated with the flour quality and increasing total protein content. Several researchers also reported the relationship between protein content and water absorption of flours (Anton et al., 2008; Collar et al., 2007; Mohammed et al., 2012). Higher DDT values indicate that the wheat flour is stronger (Meral & Doğan, 2013). In other words, addition of 0.3% CSE was more effective than 0.3% AA to strengthen the wheat flour blends. When the amount of SF increased in the blends, the strengthening effect of extract was more clearly. Although increase of water absorption of a certain flour will increase DDT levels, in our experiment, increase in DDT was not in correlated with water absorption levels. This finding was in accordance with those of the study conducted by Fu et al. (2008).

Incorporation of CEE or AA resulted in significant changes on stability and development time (DDT) of the doughs of the flour blends, as seen in Figure 1. In the case of HF, greater ($P<0.05$) stability values were observed as a result of AA incorporation as compared to CEE addition while CEE provided better ($P<0.05$) stability only for the flour sample prepared by mixing in equal ratios (50%:50%) than AA. In a study that was conducted by Karaoglu (2006) who tested farinograph properties of wheat flour doughs supplemented with whole (WCSF) or defatted *C. syriaca* flour (DCSF) and oil (CSO), lower water absorption and stability values were observed for the samples blended while mixing tolerance and degree of softening was positively affected with WCSF and DCSF levels higher than 2%.

Dough development time (DDT) is the time required to reach the point of the greatest torque. Water hydrates of the flour components is developed during the mixing phase (Fu et al., 2008). As shown in Figure 1, DDT ranged from 1.15 min to 4.50 min. For the SF, B1, B2, B3 and HF, DDT increased with increasing protein content in the flours, respectively. The flours incorporated with 0.3% of CSE had higher ($P<0.05$) DDT compared to those with 0.3% of AA. Higher DDT values indicate that the wheat flour is stronger (Meral & Doğan, 2013). In other words, addition of 0.3% CSE was more effective than 0.3% AA to strengthen the wheat flour blends. When the amount of SF increased in the blends, the strengthening effect of extract was more clearly. Although increase of water absorption of a certain flour will increase DDT levels, in our experiment, increase in DDT was not in correlated with water absorption levels. This finding was in accordance with those of the study conducted by Fu et al. (2008).

3.3. Extensograph

It is well known that extensograph analysis informs about the viscoelastic behavior of dough as a function of resting time. A combination of good resistance and good extensibility provides desirable dough properties (Rosell et al., 2001a). Six parameters were used to characterize the extensogram: Energy (cm^2), Resistance to extension (BU), Extensibility (mm),

Maximum (BU), Ratio number, Ratio number (Max). Data on the effect of incorporated CSE and AA on the extensograph characteristics of wheat flour dough samples, throughout a 135 min of resting time, are presented in Table 3. As expected, both CSE and AA incorporations made positive effects on extensograph properties of wheat flours.

Resistance to extension indicates the highest resistance level of dough during its extension, as determined by extensograph studies. Higher levels resistance to extension indicate that the dough has better processing properties and greater fermentation tolerance (Zhang et al., 2010). The resistance of the dough to extension and its extension ability are related to the gluten structure of the dough. In this study, when the effect of the extract on the resistance to extension was examined, higher resistance was observed as compared to the dough sample without CSE or AA (control). However, AA supplementation resulted in higher resistance to extension values than CSE for all flour samples. Especially as the wheat flour hardness increased, higher resistance to extension levels were observed (Table 3). This was in conformity with the results obtained by Sahari et al. (2006) who specified that resistance was affected from the protein content of flours.

Extensibility informs about the viscoelastic behavior of a dough and represents its deformation before rupture (Wang et al., 2003). A correlative combination of extensibility and resistance to extension is desired for favorable dough properties. As can be seen in Table 3, as a result of CSE incorporation to wheat flours, extensibility values ranging from 82 to 189 mm were observed. It is generally preferred in bakery to have higher extensibility in order to maintain the gas produced during the fermentation in the early stages of the pre-fermentation and cooking process. Both CSE and AA incorporation caused decrease in the extensibility values of the doughs compared to non-supplemented ones.

Energy value is an indicator of dough strength. High energy values of flours are desired in baking industry in order to manufacture more voluminous breads (Pyler & Gorton, 2008). It is well known that incorporation of oxidants increase energy value of doughs while decreasing their extensibility (Ibanoglu, 2002), as also observed by the effect of AA in this study (Table 3). Incorporation of CSE provided higher increase in energy values than that of AA for all flour samples. The results were in accordance with the findings of Boz et al. (2010) who reported that dough energy was generally increased by supplementation of rosehip, defatted *C. syriaca* flour and vital gluten.

Ratio number is the measure of the relationship between dough resistance and its extensibility. It is desired by breadmakers for flours to have a favorable combination of dough resistance and extensibility (Burešová & Hřívna, 2011; Burešová et al., 2014). In this study, CSE incorporation increased ratio numbers of the flour samples at moderate levels while the samples which was supplemented by AA had the highest ratio numbers (Table 3).

Farinograph and extensograph properties of different flours supplemented with several *C. syriaca* fractions (oil, whole or defatted *C. syriaca* flour etc.) were studied by several groups (Boz & Karaoglu, 2013; Boz et al., 2010; Karaoglu, 2006, 2012). In those studies, especially defatted *C. syriaca* flour was found to be effective to strengthen the flours while *C. syriaca* oil did not significantly affect the extensibility, maximum resistance, energy and ratio number characteristics of doughs probably due to its inability to contribute to polymeric protein structure (Bangur et al., 1997; Karaoglu, 2006).

Table 3. Extensograph properties of flour blends

Sample	Extensograph 45				Extensograph 90				Extensograph 135			
	Energy (cm ²)	RE (BU)	Extensibility (mm)	Ratio number	Energy (cm ²)	RE (BU)	Extensibility (mm)	Ratio number	Energy (cm ²)	RE (BU)	Extensibility (mm)	Ratio number
SF-C	84±5.50	438±37.50	121±0.50	3.6±0.30	82±0.50	416±12.00	126±2.50	3.3±0.20	73±2.00	333±15.00	146±2.00	2.3±0.10
SF-CSE	96±8.00	506±22.50	123±6.00	4.1±0.00	113±0.50	716±10.50	108±1.00	6.6±0.15	105±8.00	672±18.00	109±5.00	6.1±0.15
SF-AA	75±1.50	548±16.00	101±6.00	5.5±0.50	100±13.00	860±72.50	94±6.00	9.1±0.20	87±9.00	872±26.00	83±5.50	10.5±0.35
B1-C	80±1.50	348±18.50	139±2.50	2.5±0.20	78±0.50	330±2.00	147±1.50	2.3±0.05	71±1.00	306±3.00	153±7.50	2.0±0.10
B1-CSE	113±1.00	510±14.50	136±6.50	3.8±0.25	117±2.00	502±0.50	141±2.00	3.6±0.05	102±2.00	486±37.50	128±4.00	3.8±0.40
B1-AA	84±5.00	596±5.50	103±6.00	5.8±0.40	86±0.50	787±22.00	88±3.50	8.9±0.60	88±9.00	877±8.00	82±7.00	10.7±0.75
B2-C	92±4.50	338±25.50	159±4.00	2.1±0.25	80±5.50	310±12.50	167±4.00	1.9±0.05	70±1.00	254±4.50	170±7.00	1.5±0.00
B2-CSE	102±9.00	442±3.00	137±9.00	3.2±0.15	112±4.50	546±8.50	126±5.00	4.3±0.25	96±0.50	504±12.50	120±3.00	4.2±0.20
B2-AA	97±4.50	636±0.50	107±5.00	6.0±0.30	108±14.50	982±96.50	90±2.50	10.9±0.75	98±12.50	954±44.50	88±11.00	10.9±0.85
B3-C	86±2.00	311±14.00	173±10.50	1.8±0.20	81±0.00	288±8.50	173±2.50	1.7±0.05	75±1.00	271±5.00	178±2.00	1.5±0.00
B3-CSE	101±0.50	422±17.50	141±3.00	3.0±0.20	107±12.00	492±27.50	131±5.50	3.8±0.05	92±2.50	454±1.50	125±0.50	3.6±0.00
B3-AA	100±2.00	682±9.50	105±1.00	6.5±0.00	106±8.50	984±25.50	87±5.00	11.3±0.40	117±0.50	1093±12.00	88±2.50	12.4±0.50
HF-C	79±7.00	258±23.00	183±5.00	1.4±0.10	73±0.00	244±25.50	189±6.50	1.3±0.20	67±1.00	224±8.00	189±1.00	1.2±0.05
HF-CSE	100±2.00	380±1.50	156±1.50	2.4±0.05	118±4.50	450±5.00	163±5.50	2.8±0.15	104±1.50	452±12.50	139±5.50	3.3±0.25
HF-AA	103±7.50	629±27.00	114±2.50	5.5±0.10	106±2.00	926±22.50	93±0.50	9.9±0.20	120±12.00	1098±83.50	94±2.50	11.6±0.50

SF: Soft flour; HF: Hard flour; CSE: *Cephalaria syriaca* ethanolic extract; RE: Resistance to extension; BU: Brabender units; C: Control sample (without AA or CSE)

In addition, the oil of *C. syriaca* was reported to cause negative effects on organoleptical properties of the final product because of oxidation reactions (Boz & Karaoglu, 2013). On the other hand, addition of certain levels of *C. syriaca* flour gives bread a dark blue color and bitter taste. Therefore, in this study, we tested the effect of ethanolic extract of *C. syriaca* on farinograph and extensograph characteristics of flours with different protein contents in order to eliminate the aforementioned disadvantages of whole fractions of *C. syriaca*.

4. Conclusions

In this study, it was aimed to determine the effect of ethanolic extract of *C. syriaca* (CSE) on viscoelastic properties of wheat flour blends with different protein contents and quality characteristics by farinograph and extensograph studies. Ascorbic acid (AA) which is the most widely known oxidizing agent was also tested for comparison. Water absorption, development time and stability characteristics (farinograph) as well as energy, resistance to extension, extensibility and ratio number values (extensograph) were measured. As expected, AA and CSE both improved the characteristics of the flours. In terms of water absorption, extensibility and energy values, CSE provided better properties to the flour samples while effect of AA was higher in terms of development time, stability and resistance to extension. Overall, CSE provided better viscoelastic properties for the flours with different protein contents. Therefore, this study demonstrated that CSE could be used as a natural flour improver for breadmaking as an alternative to AA.

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