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BREADMAKING PERFORMANCE AND TEXTURAL CHANGES DURING STORAGE OF COMPOSITE BREADS MADE FROM SPELT WHEAT AND DIFFERENT FORMS OF GRAIN AMARANTH

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ABSTRACT

The objectives of the present study were to assess the baking properties of composite spelt wheat-amaranth blends and to study the staling of composite breads during a 6-day storage. Different forms of amaranth grains were added to spelt bread formulation: native amaranth flour and flour from popped amaranth, including their steamed and non-steamed variants. Native amaranth flour (both steamed and non-steamed) gave loaves with the highest volume and contributed to significantly softer crumb but not in comparison to the control bread. Crumb resilience did not show significant differences among the breads but there were differences in the crumb stress-relaxation parameters which indicated certain influence on the crumb visco-elastic properties.

During storage, all samples developed firmer and less elastic crumbs. Drying loss and staling degree significantly increased with increased storage time. The staling rate was the highest in the bread with non-steamed amaranth flours (native and flour from popped amaranth). The changes in the crumb textural and elastic properties caused by staling turned significant after 6 days of storage. In general, inclusion of different forms of amaranth flour did not alter the staling of breads and they exerted similar behaviour during storage.

Key words: composite bread, spelt, amaranth, staling, texture, stress-relaxation, storage, staling.

INTRODUCTION

Composite bakery products have high potentials as vehicles of functional ingredients and development of functional products. These products are based on composite flours which represent blends of wheat and non-wheat flour in appropriate proportions (Seibel 2006). Composite bakery products offer many advantages, ranging from improved nutritive profile of newly developed composite formulations to extended assortment of bakery products.

Spelt wheat is ideal for the production of organic bread because it is a low-input plant, suitable for growth without pesticides and extensive fertilization. Nutritionally, spelt wheat is similar to conventional wheat or even better, however, it is unbalanced due to lack of lysine (Kohajdova and Karovičova, 2008). Substitution of spelt wheat with other ingredients that may complement this deficiency is advisable. But, to produce bread labeled as organic, at least 95% of ingredients must be organic (Smith *et al.* 2012). Thus, ingredients feasible for organic production are highly preferred.

Amaranth has strong disposition for cultivation in organic systems (Bavec and Bavec, 2006) and has been shown in numerous works as an adequate non-wheat ingredient to be combined with wheat (Grobelnik Mlakar *et al.* 2009). Therefore, it may be suitable for composing organic composite products. Amaranth contains high quality proteins rich in lysine, high fat and iron contents. Breshears and Crowe (2013) reported that folate content was doubled in gluten-free amaranth breads in comparison to the control. Lacko-Bartošova and Korczyk-Szabó (2012) investigated the technological properties of spelt-amaranth composite flours and reported positively on the baking potential of the composite flours which was in line with the previous finding of Grobelnik Mlakar *et al.* (2009).

During storage, bread undergoes a set of severe physicochemical and sensory changes known as staling (Cauvain, 1998) that result in a loss of freshness and overall quality. The most important change associated with staling is a gradual loss of moisture and crumb elasticity, an increase in crumb firmness, loss of aroma, crumbling etc. In spite of extensive research, the mechanism of the phenomenon has not been yet resolved.

The present study was carried out to investigate the potential of various forms of amaranth in composite spelt breads. The first objective was to study the physical, textural and viscoelastic properties of composite breads as affected by incorporation of various amaranth forms (steamed and non-steamed variants of native amaranth flour and flour from popped amaranth). To the best of our knowledge, there is little data on the staling of spelt wheat-amaranth composite breads. Against this background, the second objective was to study the quality changes associated with the composite breads over a six-day storage period to see whether different amaranth forms have a tendency to ameliorate or aggravate the changes.

MATERIALS AND METHODS

Material

For the preparation ofcomposite spelt-amaranth breads, spelt flour was procured from the local ecological agricultural farm „Jevtić“ (Bačko Gradište, Serbia). *Amaranthus cruentus* grain was purchased in the local market. Popped amaranth was prepared by heating the grains on a hot plate at 200° C for 10 s.

Raw amaranth flour was prepared by milling whole amaranth grain on a Buhler laboratory mill (Buhler AG, Switzerland). The bran fraction was discarded whilst the two flour passages were combined and used in the experiment. Flour from popped amaranth grain was obtained by milling popped amaranth grains on a hammer mill Lab Mill 3100 Perten (Sweden).

Steaming of amaranth flour was performed by pouring 2 parts of hot water over 1 part of amaranth flour, stirring, covering the blend with a lid and letting it swell for 30 min.

Bread preparation

The basic formulation for the composite breads included (on flour basis): 100% spelt flour, 2.5% fresh compressed yeast, 2% salt and 0.050 g/kg ascorbic acid. In the composite breads, different forms of amaranth flour (raw flour, flour from popped grain, their steamed variants) were added at 10% level (flour basis). These ingredients were mixed according to the breadmaking procedure described in Filipčev *et al.* (2013). Final fermentation time was 55 min. Baking was performed in a deck oven at 230° C for 20 min.

After baking, the loaves were left to cool for 2 hours. The loaves were then individually wrapped up in a food-grade biodegradable perforated celophane (28 μm) and stored at room temperature. The ends were secured with a sticking tape. Though this material is not optimal solution for bread packaging, its use to pack organic, traditional and artisan breads is not unusual because, unlike polymer materials, celophane is regarded compatabile with the concept of organic food and environmental sustainability due to its natural origin and biodegardability.

Determination of bread quality attributes

Measurements of bread quality attributes were performed 24 hours after baking. Millet seed displacement method was used to measure loaf volume. Specific volume was calculated as a ratio of loaf volume and weight. Volume yield (VY) was calculated according to equation (Eq. 1):

(Eq. 1)

where V is loaf volume (ml); W_{24h} is loaf weight (g) one day after baking; BY is bread yield (g). BY is calculated as given by Eq. 2

(Eq. 2)

where DY is dough yield (g) and W_{dough} is dough weight (g).

Dough yield was calculated according to Eq. 3

(Eq. 3)

where W_{ingred} is weight of all ingredients used to make dough in g, W_{flour} used for dough making, including flour used for dusting (g).

Textural properties of bread crumb (firmness and resilience) were determined on a texture analyzer TA-Xtplus (Stable Micro Systems, England). Crumb firmness was measured in accordance to AACC (2009), method 774-10A, using a 36 mm cylinder probe. Crumb firmness is defined as the force required to compress the crumb when 25% strain is achieved. Crumb resilience was determined as a percentage of recovery of sample's height after maximal compression during 2 s followed by a recovery period of 15 s. This parameter was derived to mimic the palpatory evaluation of crumb elasticity (by pressing the crumb with fingers). Measurements were performed in six replicates.

In addition to evaluation of texture, fundamental visco-elastic properties of bread crumb were determined by collecting stress-relaxation data (Wu et al., 2012) measured on a texture analyzer TA-Xtplus. Prior to analysis, 24 mm diameter cylinders were cut out from the central part of 20 mm-thick bread slice. Test was conducted by compressing the crumb sample with a 45 mm stainless steel cylindric probe to a constant strain of 12% at 0.5 mm/s speed. The residual force was continuously recorded as a function of time during 600 s. Data extracted from the recorded stress relaxation curves were subjected to analysis using the Peleg-Normand model (Eq. 4).

——— (Eq. 4)

where F_0 is initial force, F(t) is the force at time t and k_1 and k_2 are constants. The k_1 and k_2 values are the intercept and slope of regressive straight line plotted by normalized force and time, respectively. Data were analysed in duplicates.

Determination of changes in bread quality during storage

Assessment of changes in bread crumb properties during storage was performed by determining the following parameters: drying loss, crumbliness, staling degree and staling rate.

Drying loss was measured by determining the difference in the sample moisture content after 1, 3 and 6 days of storage. Moisture content was determined according to the standard AOAC methods (2000).

Crumbliness was determined by sieving test as described in Filipović *et al.* (2009). Nine cube-shaped crumb pieces, size 25x25x25 mm, were cut out from the central part of bread slices. The crumb cubes were than sieved through a sieve with a mesh size 1.5 mm for 15 min at 190 r/min. Crumbliness was determined as the weight of throughs expressed as a percentage of the original weight of the sample.

Staling degree was defined as a percentage change in crumb hardness after the given period of storage. This parameter was calculated according to equation (Eq.5):

—— (Eq. 5)

where Ft and F1 represent crumb firmness after t time of storage and 1 day of storage, respectively.

Staling rate was calculated by regression analysis as in Sciarini et al (2010) with a modification as here the crumb firmness, measured during storage, was adjusted to exponential model where *y* denotes crumb firmness, *t* storage time and *k* staling rate constant.

Statistical analysis

One-way ANOVA was used to study the quality parameters of composite spelt-amaranth breads and their changes during storage. Honestly significant differences (Tukey's test) were calculated to differentiate between the means at significance $p<0.05$. To analyse relationships between the parameters, exploratory factor analysis (FA) was used as a tool to reveal structure in the data set and identify variables that are highly interrelated because they measure the same „construct". The analyses were performed using the Statistica 12 software (Statsoft, Inc., Tulsa, OK). Data were in triplicate unless otherwise stated.

Cluster analysis (CA) was used to classify diffrerent composite bread formulations into groups on the basis of multiple varibles (quality parameters and parameters describing changes during storage. The measure of dissimilarity between the samples was Euclidean distance and the Ward's method was used agglomerate data. XLStat software (www.xlstat.com) was used to perform the calculations.

RESULTS AND DISCUSSION

Volume and texture of composite breads

Table 1 shows volume-related parameters of breads obtained from 4 amaranth-spelt composite formulations and a control, 100% spelt formulation. Significantly higher specific volume and volume yield was determined for composite breads containing amaranth flour and steamed amaranth flour. Grobelnik Mlakar *et al.* (2008) found that, amaranth flour at 10% substitution level did not have detrimental effect on bread volume. Alvarez-Jubete *et al.* (2010) suggested that lipids in amaranth may act as surface-active agents which stabilize gas cells prior to starch gelatinization, contributing thus to higher volume. In contrast, Sanz-Penella *et al.* (2013) and Ayo (2001) observed a gradual decrease in loaf volume as a consequence of amaranth flour addition to wheat flour, but at 4-5 times higher levels of supplementation, showing the importance of the applied supplementation level. The formulations based on amaranth flour which underwent more severe hydrothermal treatment (flour from non-steamed and steamed popped amaranth) showed lower volumes. Similar findings were reported by Bodroža-Solarov *et al.* (2008) during addition of popped amaranth to wheat flour at 10-20% levels. Martinez *et al.* (2013) reported negative effects of pregelatinized (extruded) rice flour on the volume of the gluten-free breads. Dapčević Hadnađev *et al.* (2014) reported positive effects of pregelatinized starch sodium octenyl succinate (OSA) on the bread volume (5% replacement level) and attributed this to the ability of already gelatinized starch to develop dough structure and positively affect loaf volume.

Table 1. Physical, textural and viscoelastic properties of spelt-amaranth composite breads

Property	Composite spelt-amaranth breads				
		Non-steamed		Steamed	
	Control	Amaranth flour	popped Amaranth	Amaranth flour	popped Amaranth
Volume (ml)	490.0±10.6^{a}	500.8±11.7^{b}	465.0±15.8^{a}	515.0±15.0^{b}	458.3±10.6^{a}
Specific volume (ml/g)	3.92±0.1^{b}	4.00±0.1^{b}	3.49±0.1^{a}	4.09±0.1^{b}	3.47±0.1^{a}
Volume yield (ml)	354.4±9.1^{b}	368.54±6.0^{b,c}	334.1±10.6^{a}	383.6±3.4^{c}	329.7±6.0^{a}
Firmness (g)	794±63.7^{a,b}	619.81±107.8^{a}	854.98±83.2^{b}	620.33±36.6^{a}	854.64±34.8^{b}
Resilience (%)	64.02±2.0^{a}	66.58±2.9^{a}	65.12±2.5^{a}	63.70±3.0^{a}	63.01±3.0^{a}
Fmax (g)	41.73±13.2^{a}	41.67±10.1^{a}	51.61±11.3^{a}	34.88±3.89^{a}	40.91±7.19^{a}
k_1 (s)	46.72±2.46^{a}	56.28±1.17^{b,c}	59.79±4.32^{c}	43.47±2.74^{a}	49.30±1.46^{a,b}
k_2	1.94±0.07^{a,b}	1.88±0.02^{a}	2.03±0.04^{b}	1.86±0.03^{a}	1.91±0.03^{a}
%SR	50.11±1.87^{a,b}	51.44±0.67^{b}	47.76±1.15^{a}	52.32±1.02^{b}	50.67±0.72^{a,b}

^{a,b,c} Mean value±Sd.

Mean values followed by a common letter within the same row are not significantly different ($p<0.05$).

As shown in Table 1, the composite breads made with native and steamed amaranth flour exhibited a tendency toward softer crumb whereas addition of popped-amaranth flour in both steamed and non-steamed variants gave firmer crumb. However, no significant differences were noted in comparison to the control bread. Tsai et al. (2012) noted that bread containing rice porridge yielded a softer crumb than that containing rice flour and explained this as an effect of gelatinized starch in rice porridge. Steaming of amaranth flour in the present study might have produced a similar effect. Sanz-Penella *et al.* (2013), Bodroža-Solarov *et al.* (2008) and Ayo (2001) studied wheat-amaranth composite breads and observed increased crumb hardness with an increase of whole amaranth flour up to 40 g/100 g wheat flour, i.e. popped amaranth flour from 10 to 20% suplementation level i.e. amaranth flour up to 50% supplementation level, respectively, and explained this as a consequence of gluten dilution in composite flour. But, Oszvald *et al.* (2009) found that amaranth albumins are capable of interacting with gluten proteins thus improving dough strength. Moreover, high levels of fats and naturally present emulsifiers in amaranth may contribute to softer crumb (Alvarez-Jubete *et al.* 2010). Several authors reported positive effects of native amaranth flour addition to wheat flour on dough rheological properties and baking potential (Grobelnik Mlakar *et al.* 2008; Lacko-Bartošova and Korczyk-Szabo, 2012).

Popping induces similar changes to amaranth grain as extrusion; it increases water and fat absorption of the grains as a consequence of starch gelatinization and molecule fragmentation (Zapotoczny *et al.* 2006). Menegassi *et al.* (2011) observed molecular and stuctural degradation in starch granules during extrusion cooking of amaranth flour. Martínez *et al.* (2013) reported that the addition of 5% of extruded wheat flour to bread did not produce significant differences in bread quality compared to the control, though certain crumb softening effect was observed in them. Pongjaruvat *et al.* (2014) found that addition of up to 20%(replacement level) pregelatinized flour to rice gluten-free bread positively affected crumb firmness whereas higher replacement levels exerted detrimental effects. In the present study, bread with popped amaranth yielded firmer crumb and lower volume. De la Barca *et al.* (2010) noticed that popped amaranth at levels

>70% in gluten-free bread caused crumb collapse and explained this as a consequence of enhanced amaranth protein aggregation.

Crumb resilience did not show significant differences among the samples. However, the coefficients from the Peleg-Normand model showed differences: the bread with non-steamed popped amaranth flour showed significantly higher k_1 and k_2 values, indicating the most pronounced elastic nature. Another parameter, extracted directly from the stress relaxation curves, is percentage stress relaxation (%SR) and indicates the extent of relaxation. For the bread samples, it ranged from 47.8-52.3% showing that the breads are materials in which both elastic and viscous component are almost equally represented. Singh *et al.* (2006) reported similar ranges in baked products. According to this parameter, the most elastic was the bread with non-steamed popped amaranth, but significant difference existed only in relation to the bread with steamed amaranth flour.

Changes in the properties of composite breads during storage

During storage, bread undergoes many complex changes that are called staling. Bread staling is most frequently perceived as a loss of freshness which is a cumulative consequence of aroma loss, moisture migration from crumb to crust, increased crumb hardness and crumbliness, decreased elasticity, etc. Table 2 displays the most important indicators of staling (drying loss, crumbliness, staling degreee and staling rate) for the studied composite breads. Drying loss was similar for all bread samples after the first day and significantly increased after 3 days. The highest, statistically significant drying loss was registered for the formulations made with non-steamed flours (F1 and F2) after 6 days of storage. Crumbliness spanned between 9-11% on the first day of storage, and between 11-14% after 6 days of storage. During storage, crumbliness increased but without difference within the samples during storage at the same period of storage.

Staling degree significantly increased with prolongation of storage; it ranged between 60-96% after 3-day storage whereas after 3 more days, it increased and spanned between 290-580% (Table 2). The highest staling rate was presented by bread with non-steamed native amaranth flour and the lowest by the control and bread with steamed flour from popped amaranth. This coincides with the finding of Tsai et al. (2012) that bread containing rice porridge had slower rate of firming as compared to the control.

Table 2. Staling degree, staling rate, crumbliness and drying loss of spelt-amaranth breads during a 6-day storage

Bread sample	Drying loss (%)			Crumbliness (%)			Staling degree	
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	After 3-day storage	A
Control	5.80±0.47^{a}	16.61±0.87^{b}	14.73 ±1.29^{b}	10.98±1.1^{a-f}	11.04±0.75^{a-f}	13.52±1.70^{b-f}	77.28±12.68^{a,b}	30
F1	5.42±0.48^{a}	15.46±0.79^{b}	26.67±1.25^{c}	10.39±0.83^{a,b}	11.29±0.90 $^{a-f}$	14.17±1.68^{d,f}	68.01±11.39^{a,b}	58
F2	5.49±0.67^{a}	14.56±1.64^{b}	25.44±0.28^{c}	9.06±0.57^{a}	10.74±1.21^{a-d}	10.93±1.26^{a-f}	60.71±6.43^{a}	444
F3	4.56±0.20^{a}	14.19±1.49^{b}	16.55±0.56^{b}	10.48±1.06^{a-c}	12.87±1.42^{b-f}	14.09±1.35^{c-f}	96.37±5.27^{b}	35
F4	4.40±0.26^{a}	13.96±1.15^{b}	14.38±1.70^{b}	11.62±0.67^{a-f}	11.98±1.64^{a-f}	14.37±1.09^{f}	80.96±7.36^{a,b}	29

[a,b,c] Mean value±Sd; Mean values followed by a common letter within the same row are not significantly different ($p<0.05$).

F1-formulation with non-steamed amaranth flour; F2-formulation with non-steamed popped amaranth; F3-formulation with steame formulation with steamed popped amaranth

Changes in the bread texture during storage is presented in Figure 1. Significant increase in the crumb firmness was observed only after six days of storage. In this period, steamed amaranth flour bread was significantly softer than the non-steamed breads. In other storage periods, ther was no significant difference among the samples. Crumb resilience progressively decreased with storage time, however, changes were significant only after six days of storage. The bread samples did not significantly differ among each other during the same period of storage.

[Figure 1 here]

In Fig. 2, changes in the stress relaxation parameters of composite breads during storage are presented. Parameters k_1 and k_2 decreased during storage whereas %SR decreased, all indicating loss of crumb elastic properties. These changes were gradual and means significantly differed mainly between the values assessed after one day and six days of storage. An exception from the general trend was with samples containing steamed amaranth flour (native and popped) which showed a slight increase in k_1 i.e. little improvement in crumb elasticity after 6 days of storage. In general, in terms of stress-relaxation parameters, breads with steamed amaranth ingredients showed somewhat better elastic crumb properties but this was not supported by the crumb resilience values. The difference is due to the fact that different strains were used when measuring these parameters.

[Figure 2 here]

Cluster analysis

Cluster analysis was performed to reveal the overall similarity between the bread samples during storage taking into account multiple parameters: specific volume, hardness, resilience, Fmax, k_1, k_2, %SR, drying loss, crumbliness and staling degree. The resulting dendrogram is shown in Fig. 3. It can be seen that the bread samples where gathered together into 3 groups. Actually, bread samples were grouped according to the storage period showing that they were all very similar among each other at the same storage period which is supported by previous observations. The samples one day after baking were less similar than those after 3 and 6 days of storage as they were joined together at distance 0.53. The remaining amalgamation steps occured at very small distance (around 0.1) between the bread samples aged for 3 and 6 days showing they were very similar.

[Figure 3 here]

Factor analysis

Factor analysis was performed on mean values of quality attributes of the composite breads (Table 3). Factor analysis yielded 3 factors which explained 89.04% of data variation. Factor 1 explained the most variation in data (41.8%) and it was significantly loaded with parameters indicating changes in the crumb firmness (firmness, maximal force, % stress relaxation, staling degree and staling rate). Factor 2 correlated well with parameters associated with loaf volume explaining 22.6% variance of data. The third new factor explained 24.6% of the total variance and was significantly loaded with parameters related to crumb elastic properties such as resilience and stress relaxation coefficients (k_1 and k_2) but the correlation was negative. Negative loading of variable to factor is usually interpreted as an existance of opposition of variable to the factor. In this sence, it seems that the third factor is more related to plastic or viscous properties of the crumb than to elastic properties if it can be considered that plasticity is in opposite to elasticity. This finding indicates that in the spelt-amaranth bread, crumb plasticity or viscosity is a true indicator of quality changes. High loadings of coefficients k_1 and k_2 from the Peleg-Normand model and crumb resilience measured on texture analyzer showed that they essentially carry the same information. In general, it seems that quality alterations in the spelt-amaranth breads were mainly due to changes in the crumb firmness whereas changes in the crumb elasticity and differences in loaf volume contributed almost equally to the rest of the explained variance.

Table 3. Results of factorial analysis on the quality parameters of composite spelt-amaranth breads - varimax rotated loadings

Variable	Factor 1	Factor 2	Factor 3
Specific volume (ml/g)	-0.041356	**0.976012**	0.034579
Loaf volume (ml)	0.087542	**0.983452**	-0.106191
Volume yield (ml)	0.003937	**0.984076**	-0.009171
Drying loss (%)	0.650690	0.065676	0.623507
Firmness (g)	**0.896009***	-0.064815	0.415799

Resilience (%)	-0.376713	0.191235	**-0.818521**
Crumbliness (%)	0.645003	0.370730	0.174224
k1	-0.138060	-0.087575	**-0.915151**
k2	-0.347691	0.046900	**-0.868936**
%SR (%)	**0.884981**	0.227922	0.273908
Fmax (g)	**0.933265**	-0.102479	0.111621
Staling degree (%)	**0.934968**	-0.100066	0.134867
Staling rate	**0.785345**	0.088157	0.540027
Explained variance	5.852785	3.165271	3.447404
Proportion of total variance (%)	41.81	22.61	24.62

*Marked loadings are statistically significant at $p<0.05$.

CONCLUSION

The addition of amaranth in different forms to spelt wheat affected the characteristics of the obtained composite breads. The formulations with steamed and non-steamed amaranth flour exhibited higher volume in comparison to the control. There was significant difference in the crumb firmness between the breads made with amaranth flour and grinded popped amaranth regardless whether steamed or not, but none of them were different from the control bread. There was no difference in the crumb resilience within the bread formulations but the stress relaxation parameters differed for the various spelt-amaranth breads, indicating changes in the crumb elastic properties.

During storage, the composite breads underwent changes such as increased crumb firmness and loss of crumb elasticity. Drying loss and staling degree significantly increased with increased storage time. There were no significant differences within different spelt-amaranth breads at the same period of staling, except the significantly higher drying loss in the breads with non-steamed amaranth after 6 days of storage. The highest staling rate was recorded for the bread made with the addition of amaranth flour whereas the lowest rate was recorded for the control bread and bread made with steamed popped amaranth flour. Significant changes in the stress relaxation parameters were also caused mainly by storage time (between the values assessed after one day and six days of storage).

Factor analysis showed that the main indicators of quality changes in the composite spelt-amaranth breads were those related to changes in the crumb firmness. Cluster analysis supported the observation that the composite breads formulated with different forms of amaranth grain underwent similar changes during storage i.e. showed similar behaviour during storage.

It can be concluded that, in the case of composite breads packed only in perforated celophane, addition of different forms of amaranth flour did not delay nor accelerate the quality loss of stored breads due to staling. To fully recognize the potential of amaranth grains, further study is necessary which would involve the use of packaging materials with higher barrier properties and modified atmosphere packaging methods.

Declaration of conflicting interests

The authors declare that they do not have any conflict of interest with respect to the research, authorship, and/or publication of this article.

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