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# CRUMB EVALUATION OF BREAD WITH HEMP PRODUCTS ADDITION BY MEANS OF IMAGE ANALYSIS

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## Abstract

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Hemp flour composition (20–30% proteins, 7–13% fat and more than 40% saccharides) is a precondition for its usage into non-traditional cereal products. Corresponding to the fact, that hemp proteins are represented mostly by edestin, a low-molecular globulin, technological behaviour of composites containing 5–20% of hemp flour is basically different. The effect was clearly reflected in specific bread volume decrease, comparing standard wheat bread vs. wheat-hemp one. Sensorial profile of such fortified product depends on hemp sample origin, the better one was observed for dehulled wholemeal hemp flour addition. Image analysis of black-white bread cut prints revealed increasing pore densities (up to about 74%) at reversely diminishing mean cell areas (up to about 31%) for bread altered by hulled wholemeal hemp flour. Comparing to wheat standard W2, crumb appearance of bread enhanced by 5% and 20% of dehulled hemp wholemeal was described by conversely lower cell density (11 and 9 vs. 13 pores per $cm^2$) with verifiably larger cells (3.13 a 4.25 $mm^2$ against 2.35 $mm^2$). Specific bread volume and crumb penetration were significantly correlated to both cell density ($r$ –0.69 and –0.65, respectively; P = 99.9 %) and to cell mean area (0.79 and 0.69, respectively; P = 99.9 %).

wheat-hemp flour composite, technological quality, bread volume, image analysis

Image analysis method (IA) is related to materials structure description, including the food ones. Nowadays, its application field is very broad covering also meat or milling-baking industry, used there for processing failures identification. Within the cereal chemistry branch, IA serves for distinguishing of crop botanical species or e.g. wheat varieties (Fıratlıgil-Durmuş *et al.*, 2010; Zapotoczny, 2011), or for bran particles detection in flour fractions flow (Kim *et al.*, 1999). Furthermore, local bread types is possible to differentiate according their crumb structure (Brescia *et al.*, 2007), even to evaluate fluctuation in common bread quality (of rolls and baguettes; Švec *et al.*, 2011) during its producing.

The presented work is aimed at influence comparison of commercial fine and wholemeal hemp flour additions on wheat bread texture. Within the study, relationships of morphology attribute both to analytical features of tested flour composites and also to qualitative characteristics of laboratory prepared bread are described.

## MATERIALS AND METHODS

**Flour and composite samples:** flour composites were blended from two samples of commercial wheat fine flour W1, and W2 (both T650 type, industrial mill Delta Prague, production years 2010 and 2011, respectively). The former sample was combined with commercial hemp fine flour samples H1 and H3 (of conventional and bio-quality, respectively), while the latter one with wholemeal hemp ones H4 and H5 (prepared by disintegration of dehulled and hulled seed, respectively). Hemp samples H1, H4 and H5 were produced by Hemp Production CZ (Chraštice, Czech Republic) and

H3 by Hanf Natur (Lindlar Germany). Regardless to hemp type, blending rations were 95:5, 90:10, 85:15 and 80:20 (w/w) of wheat and hemp flour, respectively.

**Analytical test:** basic analytical quality was determined, following the ČSN ISO 1871 (nitrogen content according to Kjeldahl, factor 5.7; abbreviation PRO), the ČSN ISO 5529 (protein quality according to Zeleny; ZT) and finally the ČSN ISO 3039 (amylases activity estimation as the Falling Number; FN).

**Baking test and bread features**: according the internal method (Hrušková *et al.*, 2006), baking test of laboratory scale was performed. Final product, baked on baking plate, was evaluated in terms of specific bread volume (SBV), bread shape (height-to-diameter ratio; BRS) as well as 9-point sensorial profile (SEN). Three groups of features were evaluated – bread form, crust and crumb structure and taste attributes (best quality 9 points, worst one 27 points). Also bread crumb firmness as crumb penetration (PEN) with help of the penetrometer PNR-10 (Petrotest, Germany) was determined as depth of axial deformation.

**Image analysis procedure:** optimised IA procedure used was published recently (Švec *et al.*, 2011), and descriptors as bread cut area, cells per $cm^2$, mean cell area and area fraction (ratio of total cell area to measured area) were considered (BCA, #CC, MCA and AFR, respectively).

**Statistical analysis:** gained IA data was statistically pre-treated – after data row lining up, minima and extremes were excluded as upper 5% and bottom 5% of total cell number, respectively. Thus, calculated #CC, AFR and MCA means represent 90% of obtained information. For more detailed analysis of relations of crumb texture attributes and bread characteristics, pores area distribution was categorised into three classes (*Class 1*: $x < 1.0\ mm^2$; *Class 2*: $1.0\ mm^2 < x < 2.5\ mm^2$; *Class 3*: $x > 2.5\ mm^2$; adapted from Mariotti *et al.* (2013)). A correlation matrix was calculated using the Statistica 7.1 software (Statsoft Inc, USA) and it included 18 recipe variants and 17 variables (P = 95. 99 or 99.9 %).

## RESULTS

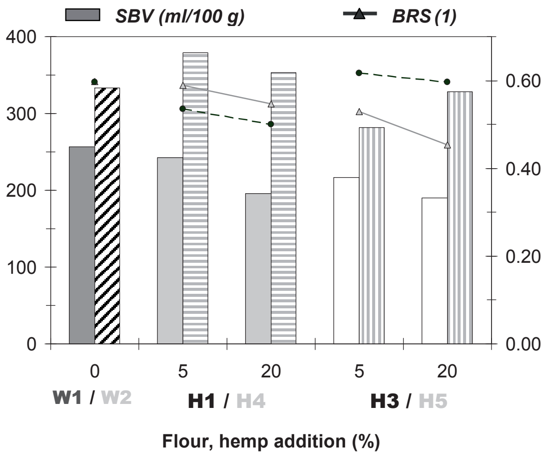
**Analytical properties of composite flour**: Both wheat flour W1 and W2 could be categorised as fine semi-bright type of standard baking quality (ash content up to 0.70%). They are characterised by average PRO (12.4%) of good quality (ZT 40 and 41 ml, respectively) with optimal values of the FN (310 and 337 s, respectively) (Tab. I). Addition of hemp flour significantly increased PRO, and reversely, protein quality as ZT was distinctly diminished independently on both hemp flour types (i.e. fine or wholemeal). By this way, a substantive part of non-gluten proteins in alternative flour, such as edestin in the hemp one, was recognized. Further differences could be find between bio-flour H3 and conventional H1 – for H3.20 composite, the ZT reached about 10 units lower value (16 ml vs. 26 ml, respectively). On the other hand, the FN values were insignificantly affected by hemp flour additions; observed changes corresponded to measurement accuracy.

**Flour composites baking test:** Both SBV and BRS of wheat bread, evaluated within a laboratory baking test, could be considered as standard; for sample W2, determined SBV reached about 30% higher value (Fig. 1). Despite comparable analytical features, higher rheological parameters were recorded for W2 (data not shown). Hemp flour additions demonstrated themselves differently in correspondence to tested type. Fine hemp ones H1 and H3 lowered SBV just at the lowest substitution level (about 20 a 15% against bread W1 volume). For samples containing H5, an initial decrease of determined SBV and then its levelling to standard W2 was observed (328 ml/100 g for H5.20 vs. 333 ml/100 g, respectively). Bread prepared from

I: *Analytical features of wheat flour and selected composite ones*

| | PRO (%) | | ZT (ml) | | FN (s) | |
|---|---|---|---|---|---|---|
| **Sample** | **average** | **SD** | **average** | **SD** | **average** | **SD** |
| **W1** | 12.49 | 0.00 | 40 | 0 | 310 | 6 |
| **H1.5** | 13.29 | 0.04 | 38 | 0 | 308 | 0 |
| **H1.20** | 14.79 | 0.05 | 26 | 0 | 297 | 4 |
| **H3.5** | 13.48 | 0.03 | 34 | 0 | 315 | 4 |
| **H3.20** | 15.93 | 0.03 | 16 | 0 | 278 | 3 |
| **W2** | 12.39 | 0.00 | 41 | 0 | 337 | 12 |
| **H4.5** | 13.27 | 0.01 | 19 | 0 | 333 | 4 |
| **H4.20** | 15.71 | 0.00 | 13 | 0 | 287 | 4 |
| **H5.5** | 12.63 | 0.00 | 34 | 0 | 333 | 3 |
| **H5.20** | 14.49 | 0.01 | 14 | 0 | 286 | 4 |

PRO – protein content (f = 5.7), ZT – Zeleny test, FN – Falling Number; SD – standard deviation.
W1, W2 – wheat flour (standards); H1, H3 – hemp fine flour, H4, H5 – hemp wholemeal flour from dehulled and hulled seed
H1.5, H1.20 – composites containing wheat: hemp flour in ratios 95:5 and 80:20 (w/w), respectively.




1: *Influences of hemp flour type and addition level on bread volume and shape (SBV, BRS)*

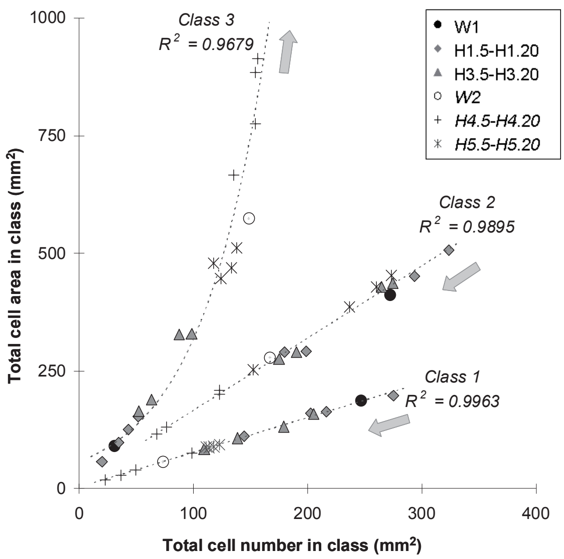
H4.5 composite reached about 14% higher volumes than one from W2 (379 ml/100 g). Further H4 dosages used manifested a negative effect; a total decrease equal to 7% is in an agreement with substitution levels (Fig. 1). Buns vaulting became stepwise worse according to composite mixing ratio, markedly within the H3.5 – H3.20 line. Sensorial profiles with narrow range (10–17 points) and penetration with very broad range (1.3–23.7 mm) was used for correlation analysis.

**IA of tested bread samples:** According to the MCA, textures of control bread W1 and W2 were different (1.382 vs. 2.346 mm$^2$, respectively), and also in the #CC (20 and 13 pores per cm$^2$). That difference corresponds with a difference in W1- ad W2-bread volumes. Understandably, such disparity seriously influenced crumb appearance of bread enhanced by fine and wholemeal hemp flour.

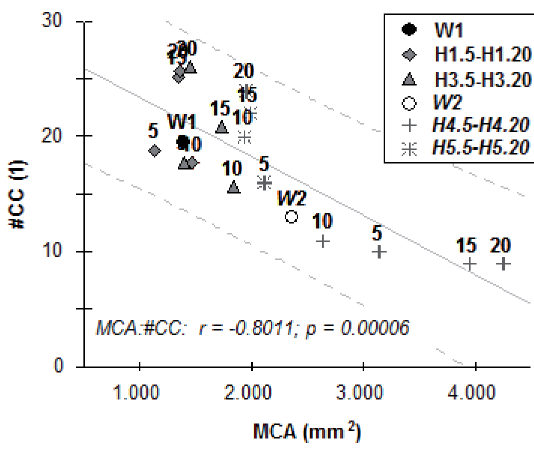
Deeper impact of H1, H3 and H5 additions on the MCA values is presented on Fig. 2 – e.g. in cases of 20% wheat flour alternation, cell densities were 26, 26 a 24 pores per cm$^2$, respectively. In general, bread crumb of composite bread was denser than one of control bread, reflected directly into different (or partially worse) sensorial profiles as well as lower determined penetration level (data not shown). Bread crumb structure involving flour from dehulled seed (H4) was vice versa characterised by comparable cell densities (10, 11, 9 a 9 pores/cm$^2$ in a samples order from H4.5 to H4.20) with almost linearly increasing MCA values (3.132; 2.641; 3.940 and 4.245 mm$^2$). Compared to W2 standard, pore size has been magnified up to twice (Fig. 2).

Pores categorisation brings an interesting view on cell size influence on bread volume.

As signify arrows on Fig. 3, only pores of the largest size (regardless to their lowest numerousness) contribute positively to bread volume (compare to Fig. 1 – higher SBV within the W2-H4-H5 subset).




3: *Relation of total cell area to cell size and to bread volume. Cell area categorisation: Class 1: x < 1.0 mm$^2$; Class 2: 1.0 mm$^2$ < x < 2.5 mm$^2$; Class 3: x > 2.5 mm$^2$. Arrows signify increasing specific bread volume.*




2: *Influences of hemp flour type and addition level on crumb morphology. MCA – mean cell area, #CC – pores per cm$^2$. Numbers identify hemp flour ratio in blend.*

**Correlation analysis of observed features:** Although in different extents, all analytical features influenced crumb appearance, at least one of the two basic attributes of crumb morphology (MCA or #CC). Diverse influence of fine and wholemeal hemp flour type affected relations verifiability. The ZT feature had the strongest links to MCA and AFR ($r$ = –0.52 and –0.69, respectively, P = 99%; Tab. IIa). Compared to analytical traits, determined bread quality macro-features (SBV, PEN, and SEN) had understandably tighter closeness to crumb texture. With an exception of statistically non-significant correlation in the SEN-AFR pair, calculated Pearson's coefficients ranged within intervals <–0.78; –0.48> and <0.50; 0.91>. As is obvious, the PEN parameter could alter the SBV one because of comparable relations tightness to MCA and #CC, which are provable at P = 99.9 %.

II: *IIa: Relations of analytical features and bread characteristics to crumb morphology parameters*

| | BCA | #CC | MCA | AFR |
|---|---|---|---|---|
| **PRO** | ns | ns | ns | 0.44** |
| **ZT** | ns | ns | –0.52** | –0.69** |
| **FN** | 0.37* | –0.48** | ns | ns |
| **SBV** | 0.90*** | –0.69*** | 0.79*** | 0.59*** |
| **PEN** | 0.91*** | –0.65*** | 0.69*** | 0.50** |
| **SEN** | –0.78*** | 0.65*** | –0.48** | ns |

PRO – protein content, ZT – Zeleny test, FN – Falling Number
SBV – specific bread volume, PEN – crumb penetration, SEN – bread sensorial profile
BCA – bread cut area, #CC – cells per cm$^2$, MCA – mean cell area, AFR – area fraction
*, **, *** – significant at P = 95, 99 a 99.9%; ns – non-significant

III: *IIb: Influence of cell size and total cell area distribution on bread quality characteristics*

| Bread characteristic | Cell number in class | | | Total cells area in class | | |
|---|---|---|---|---|---|---|
| | Class 1 | Class 2 | Class 3 | Class 1 | Class 2 | Class 3 |
| **BCA** | –0.60 | –0.48 | 0.77 | –0.61 | –0.43 | 0.76 |
| **SBV** | –0.69 | –0.67 | 0.79 | –0.70 | –0.63 | 0.85 |
| **PEN** | –0.60 | –0.59 | 0.71 | –0.61 | –0.56 | 0.76 |
| **SEN** | 0.41 | 0.59 | –0.52 | 0.43 | 0.57 | –0.55 |

BCA – bread cut area, SBV – specific bread volume, PEN – bread crumb penetration, SEN – bread sensorial profile
Cell area categorisation: *Class 1*: $x < 1.0\,mm^2$; *Class 2*: $1.0\,mm^2 < x < 2.5\,mm^2$; *Class 3*: $x > 2.5\,mm^2$.

Correspondingly to overview on Fig. 3, a positive interaction between the largest cells number or their total area and bread quality macro-characteristics (BCA, SBV, PEN, and SEN) were verified by correlation analysis through determined pore classes (Tab. IIb). Within the classes 1 and 2, calculated Pearson's coefficients were comparable for both cell number and total cells area in class as in absolute value, so in negative effect for bread quality. For links through the class 3, the highest coefficients were calculated, which were reversely marked by a positive sign. A specific position could be noticed for the SEN parameter, which stands in opposite to other observed bread quality characteristics. That fact corresponds to sensorial profile determination – the better consumer's quality, the lower value of the SEN is evaluated.

## DISCUSSION

To satisfy consumers' demand on fermented bread quality, characteristics as sufficient volume, proper vaulting as well as crumb texture must meet established conceptions of single bread types. For breads enriched by alternative flours, whose quality usually differs from common wheat bread, that perspective should be a critical point to get to like or dislike a new bakery product. Moreover, composite bread sensorial profile should be also acceptable.

Bread characteristics improvement could be reached by 20% of oat flour in recipe, as demonstrated Mariotti *et al.* (2006). Wheat-oat bread volume higher about 14% (3.7 ml/g for control bread, 4.2 ml/g for composite bread) was based on the MCA increase (about 40%, from 0.65 mm$^2$ to 0.91 mm$^2$). Enriched bread was also characterised by coarser crumb, perhaps owing to higher fat content, which supported fermentation gases capture in dough. The number of cells larger than 4 mm$^2$ increased from 30% to 45%. In terms of crumb texture, that bread should be well acceptable, but higher fat content could limit its shelf-life.

Enhancement of wheat bread recipe by 10% of barley flour led to higher mean cell area than control (2.65 mm$^2$ vs. 1.52 mm$^2$, respectively), while 10% of millet flour did not significantly changed cell size (1.48 mm$^2$; Švec and Hrušková, 2010). Both alternative raw materials have partially lowered SBV (from 343 ml/100 g to 267 and 253 ml/100 g, respectively) and increased crumb hardness (PEN diminishing from 21.0 mm to approximately a half); concluded such breads could be still accepted by consumers. Within the wheat-hemp composites set, found correlations of SBV or PEN to MCA were confirmed by these authors ($r = 0.71$ and 0.58, respectively). For bread crumb appearance with barley was obtained similar image analysis data as for wheat-hemp bread containing H4 flour. On the other hand, wheat-hemp bread texture (fortification by H1, H3 and H5) appeared similarly to characteristics of wheat-millet bread.

Addition of 40% of dehulled buckwheat flour to two commercial gluten-free premixes (GF1, GF2) resulted into significantly more porous crumb, compared to GF1 and GF2 bread texture (Mariotti *et al.*, 2013). Contribution of the largest pores to total area increased approximately from 35% up to 70%, and coarser crumb of composite bread was evaluated as firmer (thicker cell walls). Presented correlation analysis confirmed our findings – relationship of crumb hardness to middle size cells contribution to the total alveolate area was negative (r = –0.743, P = 95%), while one to the largest cells contribution to the total alveolate area was positive (r = 0.880, P = 99%).

Hager *et al.* (2012) compared gluten-free bread quality criteria for samples prepared from rice, sorghum, oat, quinoa, teff, buckwheat and maize flours with wheat and wholemeal wheat counterparts. Volume of control wheat bread was determined as the highest (2.6 ml/g), while one of wholemeal wheat was significantly lowered to 1.7 ml/g due to bran presence. For all tested non-traditional flours, a negative effect on bread volume was proved; the weakest influence was observed for oat gluten-free sample (2.40 ml/g), and vice versa for maize one (1.33 ml/g). More or less similar trends were determined also in total cell numbers, confirming its relation to the SBV. Wheat bread crumb was the most porous – 4906 cells counted within the bread slice represented 51% of slice area (equal to the AFR). Recalculated, the MCA of that wheat control bread was 0.72 $mm^2$. Other tested recipes led to decrease of numbers of cell, caused firstly by bran in wheat wholemeal (2453 cells, 55% of slice area; MCA 1.07 $mm^2$) and further by lack of gluten in alternative flours. Teff bread slice contained 3327 cells which covered 48% of slice area (MCA 0.63 $mm^2$), and the lowest cell number was evaluated for rice bread (2507 cells, AFR 54%, MCA 1.14 $mm^2$). As could be noticed, non-traditional flour impact on crumb morphology was different among used samples, and a reversal relationship between pores density and the MCA was also verified by Hager *et al.*

Summarised, non-traditional raw materials used nowadays in cereal chemistry are characterised as non-gluten ones, which lower technological quality of their composites with wheat flour. Influence of such flours from quinoa, buckwheat or rice on bread quality and texture is well documented, but for hemp products application, no relevant reference in recent scientific literature was found. In the case of other non-traditional cereals bread fortification (Švec and Hrušková, 2010), four archaic wheat species (TSP – *Tritium spelta*, TDI – *T. dicoccum*, TRI – trinaldina 'Abissinskaja arrasajta', WPG – wheat with purpur grain) were evaluated. In case of samples originated in year 2006, bread crumb appearance got better by TSP and TDI addition – MCA increased twice (2.2 and 3.0 $mm^2$ against 1.5 $mm^2$ for the control). Number of cells/$cm^2$ was in that year significantly increased by TRI and WPG (60%) as well as by SBA fortification (40%). To compare with our results, similar image analysis data was obtained at evaluation of wheat-hemp bread containing H4 flour.

## CONCLUSION

Owing to their nutritional composition, hemp seed and flour finding nowadays a new application in food industry. Fine hemp flour in blend with other types, especially wheat one, demonstrates different dough technological properties as a response of globulin proteins presence. Model flour composites involving from 5% to 20% of hemp flour are characteristic by Zeleny value decreases. However, chemical stage of starch is not significantly affected by variations in blends composition. The higher hemp level, the lower specific bread volume in case of fine hemp flour type testing. Reversely, a slow increase occurs for wholemeal hemp bread volumes. Correspondingly to that, bread crumb texture also changed – in the latter case, pores density increased and their size softly decreased; in the latter one, mean cell area was magnified but cell numbers were kept on a similar level. Exploration of cell size influence on bread quality was closed by finding of positive interaction between the largest cells and the highest specific bread volume.

## SUMMARY

Wheat-hemp blends prepared from wheat flour W1 and commercial hemp fine flour samples H1 and H3 as well as ones from wheat flour W2 and hemp wholemeals of dehulled and hulled type (H4 and H5, respectively), contained 5, 10, 15 and 20% of alternative flour.

By hemp additions, protein content was increased up to about 27%, but its baking quality was diminished about 60% due to gluten net dilution by hemp prolamins. Hemp flour portions partially changed starch properties (described by Falling Number), but observed lowering was insignificant in terms of measurement accuracy.

Comparing baking test results between wheat W1 and W2 standards, specific volume was about 30% lower for wheat bread from W1 flour (257 and 333 ml/100 g, respectively). Also crumb texture of the former bread was analysed as less porous (mean cell area 1.3 $mm^2$ and 20 pores per $cm^2$ vs. 2.3 $mm^2$ and 13 pores per $cm^2$, respectively). Hemp samples H1, H3 and H5 caused somewhat denser crumb (i.e. a partial mean cell areas lowering and cell numbers increase), only H4 improved crumb morphology (mean cell area 4.2 $mm^2$ and 9 pores per $cm^2$) due to higher fat content in hemp wholemeal from dehulled seed.

The results of presented experiment verified a dependence of bread quality parameters (specific volume, bread cut area, crumb penetration) on determined crumb morphology attributes. According to mean cell area, pores were selected into three classes: $x < 1.00$ $mm^2$, $1.00$ $mm^2 < x < 2.50$ $mm^2$ and

x > 2.50 $mm^2$ for each of samples studied, and within the single class, cell number and total cell area were calculated. The detailed analysis proved positive relationships of bread cut area, specific bread volume as well as crumb penetration for the cell number and total cell area within the class of the largest cells only ($r$ from 0.71 to 0.85, P = 95%).

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