

French Bread Loaf Volume Variations and Digital Image Analysis of Crumb Grain Changes Induced by the Minor Components of Wheat Flour

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ABSTRACT

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A standard quality flour for French breadmaking was fractionated by extraction of water-soluble components (6% db) and by defatting (<1% db) to study the impact of soluble components and lipids on bread quality in terms of loaf specific volume (v_s) and crumb structure. Addition of puroindolines (<0.2%) was also tested. Crumb cell structure was assessed by digital image analysis (DIA) according to erosion-dilation and closing treatments. The fraction of cells area with size <1 mm (%d<1) was defined as an index of fineness of crumb structure. Both DIA procedures allowed differentiation of crumb structures obtained by various formu-

lations and, in the range of composition modifications tested, variations by a factor of 2 of both criteria (v_s and %d1) were obtained. Soluble fraction increased v_s and decreased fineness. Defatting and adding puroindolines increased fineness with no effect on v_s . The possible role of molecular components of each flour fraction was discussed in terms of rheological and foaming properties. DIA methods and flour recipes tested in this work offer a valuable tool for further studies on the processing-structure-properties relationships of French bread dough and crumb.

Wheat is a unique cereal; when mixed with water, the flour gives a viscoelastic dough that retains gas during mixing and fermentation and in the early stage of baking (He and Hosney 1991). This remarkable property has given rise to the diverse and worldwide use of wheat-leavened products. Because gas retention and expansion throughout the breadmaking process determine bread volume and crumb texture, their control is still a daily challenge for the baking industry. Although crumb texture encompasses sensory perception through mechanical properties, it is often defined for bakery products as the exposed cell structure of crumb when a loaf of bread is sliced (Kamman 1970).

Differences in the baking properties of wheat cultivars primarily depend on both protein content and composition of gluten proteins (gliadins and glutenins) (Shogren et al 1969) relative to total protein content (Mac Ritchie 1987). The composition of glutenins is also essential because a higher proportion of high molecular weight glutenin subunits (up to 18% of total proteins) increases loaf volume and improves dough rheological properties, leading especially to an increase of tolerance to overmixing (Weegels et al 1996). The influence of minor flour components such as lipids, lipid binding proteins, and water-soluble molecules has also been investigated. These components are involved in the formation and stability of gas-water interfaces of wheat dough (Marion et al 1998). Polar lipids, such as phospholipids and glycolipids decrease the loaf volume until a threshold concentration is attained. Above this concentration, the loaf volume increases (MacRitchie and Gras 1973). These observations were interpreted as competition between the surface-active soluble proteins and polar lipids for the gas-water interface, followed by the progressive replacement of the interfaces by polar lipids (Gan et al 1995). On the contrary, increasing the nonpolar lipid content of triglycerides and free fatty acids leads to a continuous decrease of bread loaf volume because these lipids provide a new interface for surface-active components dispersed in the aqueous phase of dough and are also capable of destabilizing the interfacial protein films through a Marangoni effect (Marion et al 1998). In this regard, it is interesting to emphasize the effect of puroindolines, the major lipid-binding proteins from wheat, on the crumb texture (Dubreil et al 1998). This effect was attributed to the foaming properties of this

protein component and to its ability to prevent the destabilization of protein foams by lipids (Dubreil et al 2002). Water-soluble components (WS) have no significant effect on loaf volume when they are added to a standard wheat flour, but they may contribute to gassing power and rheological properties of the gluten-dough matrix (Hosney et al 1969). No effect of WS on crumb texture has been reported yet. Among WS, the role of pentosans is of particular interest due to their beneficial influence on breadmaking (Courtin et al 1999), which can be interpreted either by their effect on gluten formation or their interactions with nonpolar lipids (Wang et al 2002).

Most of these studies have used microscale baking tests and white pan bread recipes including sugar, shortenings, and eventually nonfat powdered milk and malt syrup. The traditional French bread recipe is different and simple because it contains only flour, water, yeast, and salt. Baking tests for French bread are very sensitive to wheat flour as underlined, for instance, by the influence of protein content and composition on baking properties (Tronsmo et al 2002). Lower mechanical work input is required for dough mixing than for white pan bread manufacturing. Moreover, the specific volume of French bread is generally lower than that of pan bread and it displays a crusty surface and a heterogeneous crumb structure with numerous large gas cells (Baardseth et al 2000).

Methods for an objective description of crumb texture have been developed based on digital image analysis of sliced bread, recorded in 256 gray levels. Techniques based on cell segmentation, leading to binary images, by thresholding and edge detection have been used to produce data that give a physical understanding of crumb grain features. The threshold gray level can be either subjective (chosen by experimenter) or objective (based on a mathematical function). Sapirstein et al (1994) applied the K-means algorithm to determine a gray level threshold that has to be optimized for each bread type to take brightness variation into account. Differences between characteristics such as mean cell size, cell density (cm^{-2}), average cell wall thickness, and cell total area fraction (void fraction) could be determined for standard and oxidized breads. Zghal et al (1999) used this method to determine the influence of mixing treatment and proofing time on these variables for two different wheat flours and showed that bread crumb density could be predicted from cell wall thickness and void fraction parameters ($r = 0.89$). In bread scoring, Zghal et al (2001) defined crumb fineness by cell density and later defined grain uniformity as the ratio of smaller to larger cells. From the gray level frequency histogram, Crowley et al (2000) applied thresholding on each digitalized image of white bread to study the influence of fat and emulsifier additions on similar bread charac-

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teristics. Finally, one main use of digital image analysis using cell segmentation is to help to predict the mechanical properties of bread using models for cellular solids and thereby optimize processing and formulations (Scanlon and Zghal 2001; Zghal et al 2001).

Conversely, using image texture analysis methods, data extracted from the processed image can be directly used for scoring purposes by assessing the general appearance of crumb (visual texture) rather than by extracting cells. Bertrand et al (1992) found that six texture characteristics from two-dimensional Haar transform processed images could correctly identify 82% of bread crumb images. By analyzing subimages of 64 × 64 pixels, Zayas (1993) developed a ranking scale to evaluate the degree of coarseness of crumb grain in a slice. Applying a method based on the Fourier transform to standard scanned images, Rogers et al (1995) characterized crumb fineness and cell elongation. Loaf volume was estimated from a calculated slice area and loaf length ($r^2 = 0.97$) and cell fineness were compared with crumb scores. Kvaal et al (1998) compared different methods of texture analysis to extract sensory properties of bread. Although helpful for overall characterization, results from texture analysis are difficult to interpret because their physical meaning is not clear.

With techniques based on mathematical morphological treatment, it is possible to keep all the textural information present in images, avoiding limitations linked to thresholding, together with providing a granulometric approach. The two basic operations for morphological treatment, erosion (removal of edge touching pixels to features) and dilation (adding of edge pixels to features) lead to a so-called size distribution curve (Soille 1999). For instance, particle size analysis was successfully applied to a mixture of steel marbles of three different sizes with a good correlation ($r^2 = 0.95$) between median parameters measured by laser diffraction and the first principal component of the granulometric curve (Devaux et al 1997). To our knowledge, this technique has not been used yet to characterize bread crumb.

Therefore, the purpose of this work was to use particle size analysis to study the role of minor wheat components such as lipids, water-soluble components, and puroindolines on the texture of French bread crumb.

MATERIALS AND METHODS

Wheat Flour

The standard commercial bread flour, Corde Noire Special (CNS), provided by Moulins Soufflet (Nogent, 10-France) contained 10.5% protein (db), 13.8% water (wb), and 0.56% ash (db). Alveograph measurements (Chopin, Trappes, 78-France) gave W and P/L values of 194 J and 0.56, respectively, in agreement with standard French breadmaking procedures.

The corresponding defatted flour was obtained by gently stirring the flour with methylene chloride (1 kg/2 L) for 1 hr at room temperature. The slurry was filtered through a Büchner funnel and the procedure was repeated twice. The solvent was evaporated by resting the flour for at least 12 hr at room temperature and at atmospheric pressure under a fume hood. The choice of the organic solvent methylene chloride is essential to maintain the functional properties of gluten proteins while most of the total nonstarch lipids are extracted (MacRitchie 1981; Gan et al 1995). The lipid content of nondefatted and defatted flour is determined by gas chromatography after transmethylation of wheat lipids according to the procedure described by Welch (1977). Defatted flour still contained 0.62% lipids (db), corresponding mainly to the tightly bound lipids within starch granules.

Fractionation of Wheat Flour

Flour (1 kg, defatted or not defatted) was stirred with 4 L of distilled water at 4°C for 30 min. After centrifugation at 14,000 × g, at 4°C for 30 min, the pellet F1 (insoluble components, mostly gluten and starch) and the supernatant F2 (soluble components)

were recovered, frozen, and freeze-dried. Freeze-dried fraction F1 was ground through a 250- μ m sieve. On a dry basis, F1 and F2, represented 94 and 6% of total CNS flour, respectively. F2 was dialyzed against water using dialysis tubing with a cut-off of 1,000 Da, to separate soluble components of very low molecular mass (F2.2, sugars and minerals) from those of higher molecular mass (F2.1, mainly proteins and soluble pentosans). Both fractions were freeze-dried and stored in plastic flasks at 4°C. The procedure and fraction nomenclature are represented in Fig. 1.

Puroindolines (PIN) were purified using Triton X-114 phase partitioning and cation exchange chromatography as described by Dubreil et al (1997). The crude PIN fraction was dialyzed against water, freeze-dried, and stored at 4°C. It contained more than 95% PIN with \approx 80% PIN-a and 20% PIN-b as assessed by ELISA.

Biochemical Analysis

Moisture content was determined by mass difference after oven drying (AACC Approved Method 44-15A; NF ISO 712). Protein concentration in flour and fractions was determined by the Kjeldahl method ($N \times 5.7$ on a dry basis). Lipid concentration was determined by gas chromatography after methylation of fatty acids as described by Welch (1977). Low molecular mass sugar content was determined using the Dionex system (HPAEC-PAD). Puroindoline content was determined by ELISA as described previously (Dubreil et al 1998). SDS-PAGE was performed according to the procedure described by Laemli (1970).

Dough Mixing and Baking

Flour (200 g), water (126 g), yeast (5 g), and salt (4.4 g) were mixed in an alveograph mixer for 2 min at 40 rpm and 13 min at 80 rpm. Salt was added 5 min before the end of mixing. Before mixing, fraction F1 was equilibrated \approx 12 hr at 20°C and 75% rh to prevent any water adsorption or partitioning on mixing. Water was added to the dough to get a constant hydration at the end of mixing for all doughs. After mixing, doughs were rounded by hand and allowed to rest at 27°C, 75% rh for 45 min. Then they were divided into three pieces of 80 g each and mechanically rolled. Small cylinders of dough (\approx 15 cm length and 2 cm diameter) were rested at 27°C and 75% rh for 90 min. Before baking, a cut was made with a blade at the surface of the rolled pieces of dough to orientate dough expansion during oven spring and to produce final scars on the crust, which are characteristic of French bread.

Six pieces of dough were carefully placed in an electric oven (Bongard, Wolfisheim, 67-France, P = 10.5 kW, volume = 110 L)

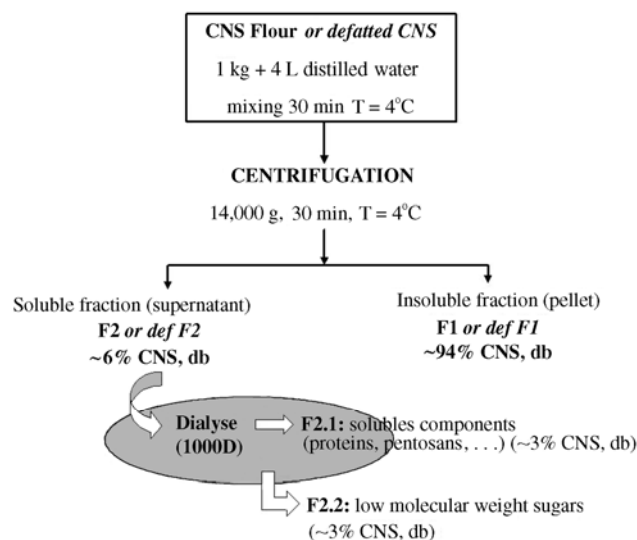


Fig. 1. Fractionation of commercial bread flour (CNS) and defatted CNS by centrifugation and dialysis.

and baked for 23 min under conditions described in Sommier et al (2004). Upper and lower plate (vault and hearth) temperatures were set at 235 and 265°C, respectively. After cooling for 90 min, bread was weighed and its volume measured by rapeseed displacement. Twelve hours after baking, three slices of bread were cut in the middle of each bread, and monochrome images were recorded with a digital CCD video camera (Sony Media Vision). The field of view was a rectangle 10.3 × 13.3 cm² (576 × 768 pixels) and spatial resolution was 177 μm. Gray levels were coded from 0 (black) to 255 (white).

Image Analysis

Visual texture of bread crumbs was evaluated by two granulometric methods using gray level mathematical morphology (Soille 1999; Aubert and Jeulin 2000). Crumb images were considered to contain gray level information from pixels of which the darkest individuals belong to cell and the brightest belong to cell wall.

Erosion-dilation method. The objective of the first method, based on the two basic morphological operators (erosion and dilation), is to characterize both cell and cell wall sizes. A structuring element of given size and shape, usually a square of $(2n + 1)^2$ pixels with a reference pixel at the center, is moved through all image pixels; n being the step of the transformation. In erosion, the reference pixel is given the minimum value met by the structuring element. The effect is to decrease the size of clear objects larger than the size of the square and to remove those objects for which at least one dimension is less than $(2n + 1)$. Figure 2 shows raw and processed images of a bread slice to illustrate how the finest details of the walls were removed by erosion. After the erosion step, the sum of all gray levels, called the image volume, decreases depending both on the amount and size of bright objects. Dilation is a dual operation, where the reference pixel is

given the maximum value met by the $(2n + 1)^2$ square; bright objects are dilated and dark objects (gas cells) decrease, as shown by the image associated with dilation step 5 (Fig. 2). In the meantime, the sum of gray levels increases. A volume evolution curve is obtained by plotting the sum of the gray levels, from the last dilation step value to the first one, and from the first one to the last erosion step, respectively. The resulting image volume curve is normalized by:

$$g(n) = |(G(i) - G(N)) / (G(0) - G(N))| \quad (1)$$

where n is the dilation or erosion step; $G(0)$ is the volume of the initial image; and $G(N)$ is the volume of the last erosion or dilation step; and N is the total number of steps (25 in the present case). Variations of first derivative of $g(n)$ are assessed separately for erosion and dilation, respectively, and then plotted together as erosion-dilation (ERDIL) or granulometric curves (Fig. 2). As is characteristic of the texture of the initial image, the left side (dilation) gives information on cell size, whereas the right side (erosion) reflects cell wall thickness.

Closing method. This method characterizes the cell size more specifically. It consists of a dilation step immediately followed by an erosion step of the same size. Like a sieving operation, the effect is to remove the dark objects smaller than the structuring element and to preserve the general size of larger ones. The increase of image volume measures the gray level fraction of dark objects (cells in our case) that have disappeared. For erosion-dilation curves, the variations of the image volume is normalized according to the initial volume. Applications of closing steps, using Equation 1, where n is the closing step, defines the size of the structuring element, resulting in a particle size distribution curve. For $n = 3$, the gray level fraction of cells calculated is denoted as %d1; this is the fraction of cells with a size <1 mm and is used to define the fineness of the crumb cell structure.

Pretreatment and normalization procedure. Starting from the raw image, as shown in Fig. 2, the region of interest (ROI) was selected by first extracting crumb from background and then eliminating crust. The image treated was always within an entire slice of bread. Crumb areas were preprocessed to normalize gray levels over the surface and between images. A dilation of size $N+1$, N being the maximum erosion and dilation steps, was applied to estimate the shading over the crumb area (Tomazevic et al 2002). Images were divided by the dilated images and the gray levels were set between 0 and 255. An histogram equalization step finally achieved the normalization procedure. Erosion-dilation or closing treatments were performed on at least three slices for two different breads. Variation of the sum of grey levels for a given dough composition was <10%, although a higher precision may be obtained for the first steps of treatment. Software was developed within the Aphelion v. 3.1 (ADCIS-SA, Herouville, 14-France) and Matlab v. 6.5 (The Math Works, Sevres, 92-France) programming environments for image processing and data treatment.

RESULTS AND DISCUSSION

Fractionation

The overall biochemical composition of the CNS flour and fractions are given in Table I. Protein content (10.5% db) is rather lower than values usually encountered for white pan bread (Pylar

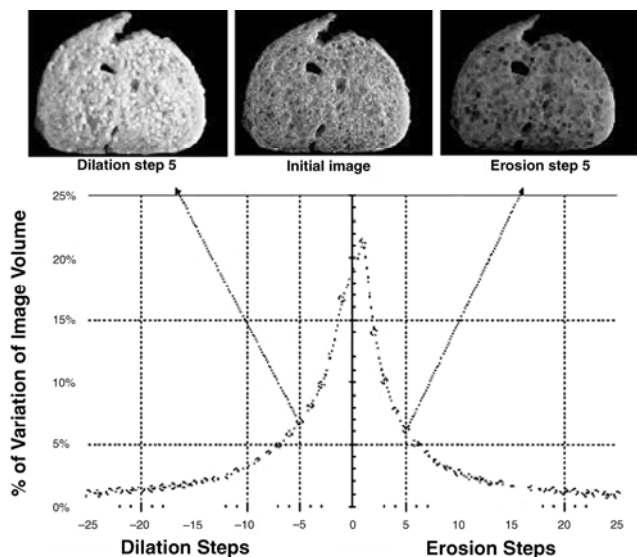


Fig. 2. Illustration of the principle of erosion/dilation treatment, by the initial image and those obtained after two treatment steps, and by the curve of variation of image volume vs. erosion (positive) or dilation step (negative) (ERDIL curve).

TABLE I
Biochemical Composition of Fractions^a

Sample	Starch	Proteins	Lipids	Pentosans	LMW Sugars	PIN	Ash
CNS (def CNS) native flour (defatted flour)	80	10.5	1.6 (0.62)	1.6	2.6	0.104	0.56
F1 (def F1) insoluble fraction	nd	9.9	1.61 (0.62)	1.1	nd	0.079	nd
F2 (def F2) soluble fraction	nd	19.2	0.48 (0.11)	6.9	17.9	0.044	nd

^a Composition in g of component/100 g of fraction, db; nd, not determined.

1988). This difference for French bread has to be kept in mind when comparing experimental results to those from other works. Conversely, lipid, pentosan, and puroindoline contents are representative of standard commercial mixtures of wheat cultivars (Guinet and Godon 1994; Dubreil et al 1998). As expected, the overall biochemical composition of def CNS differs from its parent flour by its lipid content. About half the total lipids are removed by methylene chloride. Residual lipids should correspond mainly to lipids tightly bound within the starch granule and to a lesser extent to nonstarch lipids tightly bound to proteins (Morrison 1976).

Centrifugation of wheat flour provides two fractions, F1 representing 94% of dry flour, mainly composed by starch and gluten, and F2, 6% of dry matter, composed of water-soluble proteins, mainly albumins and low molecular mass sugars. Although not determined, wheat flour minerals are probably concentrated in this fraction. Defatting does not modify this distribution. SDS-PAGE of fractions F1 and F2 are characteristic of gluten and albumin-globulins, respectively (Fig. 3). In line with this result, dialysis of soluble components underlines the presence of pentosans and albumins in F2.1, whereas F2.2 contains mainly low molecular mass sugars and wheat flour minerals. While the accurate composition of all fractions was not determined, it is sufficient to highlight the enrichment in minor components of F2 and F2.2.

Most of puroindolines are recovered in the insoluble fraction of wheat flour as shown by ELISA (Table I). This partition of PIN-a is not surprising with regard to the localization of PIN-a in the starchy endosperm of wheat kernel (Dubreil et al 1998) and their strong lipid binding properties (Dubreil et al 1997). In this regard, it is worth noting that a similar partition of lipids and PIN-a between F1 and F2 is obtained for the defatted fractions (def F1 and def F2), probably because PIN interact with starch granules (Bloch et al 2001) and the residual nonstarch polar lipids, which are not extracted with the nonpolar methylene chloride (Dubreil et al 2002).

Baking Results

Baking data are provided in Table II. As expected from the breadmaking process and recipe used in this study, values of specific volumes ($v_s < 5 \text{ cm}^3/\text{g}$) are in agreement with the values encountered for French breads (Sommier et al 2004) and rather lower than those observed for pan breads, for which values of 5–9 cm^3/g were reported by Weegels et al (1996) and Zghal et al (1999).

Breads obtained from defatted and nondefatted CNS flour have a specific volume of 4.2 cm^3/g . The specific volume (3.7 cm^3/g) of baked reconstituted doughs (94% F1 + 6% F2) is significantly lower than v_s of baked CNS dough. This difference could be related to changes in the partition of water among the components on mixing. In this regard, it is worth noting that freeze-drying of CNS flour gives rise to lower specific volumes (3.9 cm^3/g), which are close to those of baked reconstituted doughs. Therefore, the results obtained after addition or removal of the different fractions have to be related to those obtained for the baked reconstituted doughs (94% F1 + 6% F2).

Lowest specific volume (2.6 cm^3/g) is obtained for doughs from which soluble fraction F2 is removed (100% F1), whereas reconstituted doughs with defatted F1 and water-soluble F2 give the largest specific volume (4.6 cm^3/g). These significant variations delimit an experimental range large enough to examine the

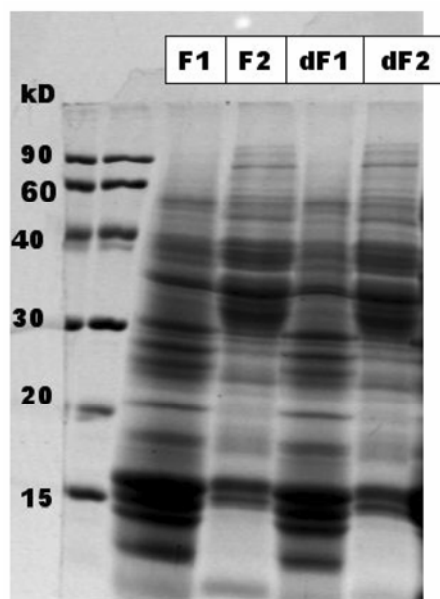


Fig. 3. SDS-PAGE of proteins from flour fractions separated from commercial bread flour (CNS) (F1, F2) and defatted flour (def F1, def F2).

TABLE II
Different Compositions and Results of Specific Volume (v_s) of Breads and Crumb Fineness

Bread Dough Composition	Number of Slices	v_s (cm^3/g)	CI (95%) (+/- cm^3/g) ^a	Fineness (%d1)
CNS flour	30	4.2	0.1	38
Freeze-dried CNS dough	6	3.9	0.3	nd
Defatted CNS (def CNS)	10	4.2	0.2	53
100% F1	6	2.6	0.3	58
97% F1 + 3% F2	6	2.7	0.2	49
94% F1+ 6% F2 (reconstituted dough)	13	3.7	0.2	44
91% F1 + 9% F2	6	3.8	0.3	41
88% F1 + 12% F2	6	4.5	0.3	33
97% F1 + 3% F2.1	6	3.7	0.2	47
97% F1 + 3% F2.2	3	2.7	0.4	43
95.5% F1 + 1.5% F2.1 + 3% F2.2	3	2.7	0.3	50
95.5% F1 + 3% F2.1 + 1.5% F2.2	3	2.9	0.1	51
94% F1 + 3% F2.1 + 3% F2.2	3	3.1	0.6	41
91% F1 + 3% F2.1 + 6% F2.2	3	4.0	0.3	38
91% F1 + 6% F2.1 + 3% F2.2	3	3.5	0.2	43
100% def F1	3	3.3	0.2	56
def (94% F1) + def (6% F2)	3	4.2	0.3	56
94% def F1 + 6% F2	3	4.6	0.2	57
94% F1 + 6% def F2	3	4.0	0.2	40
CNS flour + 0.1% PIN	3	3.6	0.6	44
CNS + 0.2% PIN	3	3.9	0.3	51
def CNS + 0.1% PIN	3	4.0	0.5	58

^a *, Value at the 95% confidence interval.

effect of each fraction on specific volume. When increasing soluble fraction F2 (100% F1 to 88% F1 + 12% F2), specific volume (v_s) increases by a factor of 1.7. This effect could be attributed to increased yeast activity in the presence of a higher sugar content (Table I). The increase of v_s from 3.1 to 4.0 cm^3/g when increasing the content of the sugar-rich fraction (F2.2 from 3 to 6%), other soluble components (F2.1) being constant (3%), agrees with such an interpretation. However, the v_s value of 3.1 cm^3/g is significantly lower than the value of the reconstituted flour (F1 + F2, $v_s = 3.7 \text{ cm}^3/\text{g}$). Moreover, the value found when sugar content is decreased to a minimum by suppressing F2.2 (97% F1 + 3% F2.1) is also 3.7 cm^3/g . Removal of the other soluble fractions (F2.1 pentosans and soluble proteins), while adding sugars to the initial level (97% F1 + 3% F2.2) leads to a much lower v_s value (2.7 cm^3/g). These results show that both sugars and other soluble components (pentosans and proteins) have positive but nonadditive effects on loaf volume. It may be suggested that the impact of soluble components relies as much on the changes of dough rheological properties, due to the polymers contained in F2.1 (albumins and pentosans), as on an increase of gas production due to the low molecular mass sugar content, mostly contained in F2.2.

Defatting of any fractions leads to an increase of v_s , if reconstituted dough (F1 + F2) is chosen as a reference. Reconstituted defatted dough (def F1 + def F2) has the same v_s as defatted CNS (4.2 cm^3/g), and the largest volume is observed when F2 (6%) is added to def F1. This result suggests that lipids present in the insoluble fraction F1 have a negative effect on bread volume, while the positive role of the soluble fraction on loaf volume is confirmed. Although the polar lipid content has not been determined, these results are in line with the effects of added lipids on bread volume (MacRitchie 1981) because polar lipid content is <1%, whatever the reference dough considered, F1 or (F1 + F2). Table II also shows that, as a general trend, addition of puroindo-

lines slightly decreases specific volume of CNS dough, defatted or not. This result has already been observed when PIN are added to a PIN-a free flour (Dubreil et al 1998).

Crumb Texture Evaluation

Typical images of bread slices are shown in Fig. 4. Normalization treatment further limits the effects of different coloration due to illumination and contrast. No attempt at this stage has been made to evaluate loaf volume by image analysis, but these images already reveal significant differences in the cell size distribution of crumb: F1 + 12% F2 bread has a coarse grain, whereas F1 and def CNS + 0.1 PIN have a much finer one. Both erosion-dilation and closing treatments were performed on all breads slices.

Erosion-dilation (ERDIL) curves provide an accurate view of these changes. Five to six curves are analyzed for each composition but only one curve is presented in Fig. 5. The lower the level of F2 fraction, the narrower the curve peak; F2 fraction modifies the crumb structure on both cell and cell wall sizes. Starting from F1 up to 12% F2, there is a continuous marked decrease of number of smaller cells, and a concomitant increase of the number of larger ones. Curves cross over for step 3, which suggests, according to image resolution (177 μm) and the ERDIL procedure, that cell size has a threshold value of $\approx 1.24 \text{ mm}$. Conversely, a similar trend is observed for cell wall thickness; a larger content of soluble fraction leads to thicker walls (>1.24 mm), whereas bread slices from dough with lower content of F2 display thinner walls. In other words, crumbs with a higher number of smaller cells have thinner walls, whatever the loaf specific volume, according to the amount of soluble fraction added. Similar opposite relationships between number of cells and cell wall thickness is found for pan breads from different wheat flours and bread-making conditions (Zghal et al 1999, 2001). Values of cell wall thickness encountered in these studies range in the same order of magnitude, although slightly lower (0.7 mm), which is in line with the finer crumb of pan bread when compared with French bread (Baardseth et al 2000).

Other ERDIL curves lead to similar trends, whatever the bread composition, fraction F2 having the most significant effect. Defatting and PIN addition generally lead to smaller cells with thinner walls. While bringing information on cell wall thickness and confirming visual observations of cell structure images, this technique also allows classification of slices from different compositions. Although beyond the objective of this study, principal component analysis may be performed on ERDIL curves for this purpose. The first principle component has generally a high inertia (80%) and is directly correlated to average cell size, as in particle analysis (Devaux et al 1997).

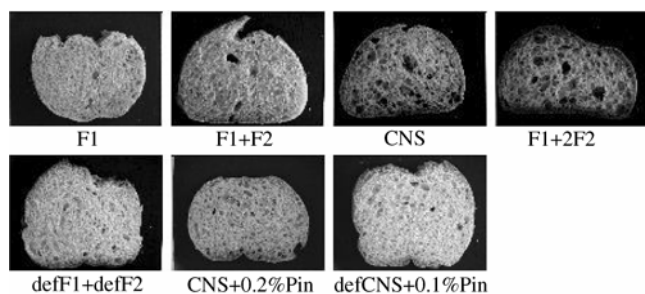


Fig. 4. Photographs of bread slices for different dough compositions before normalization.

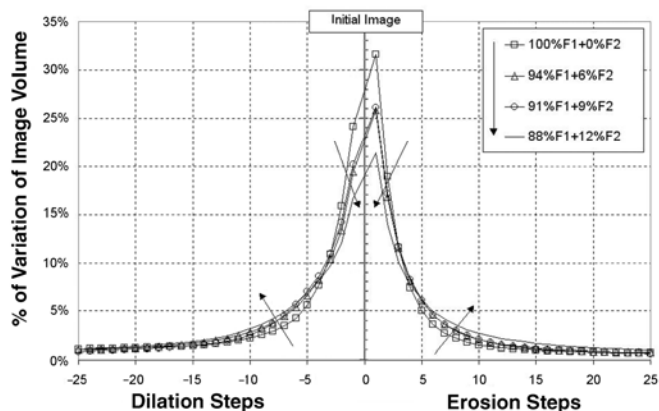


Fig. 5. Erosion-dilation (ERDIL) curve for different reconstituted doughs: effect of adding soluble fraction F2 to insoluble fraction F1.

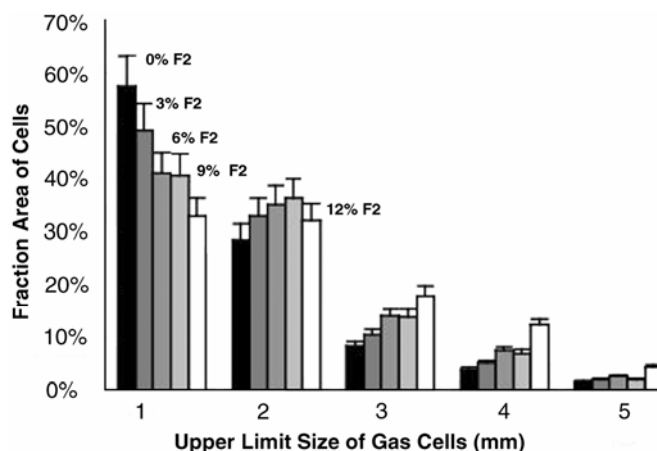


Fig. 6. Distribution of gas cells area fraction for different reconstituted doughs: effect of addition of soluble fraction F2 to insoluble fraction F1 (from left 0% to right 12%).

Although not providing information on cell wall thickness, closing treatment allows us to assess more directly the differences of cellular structure, as illustrated in Fig. 6. Increasing F2 from 0 to 12% continuously decreases the fineness of the crumb (%d1), from 58 to 33%. In the meantime, all fractions of cells >2 mm in size increase at least by a factor of 2. This result is consistent with ERDIL curves, for which a threshold size value for crumb cell is close to 1.24 mm (Fig. 5). This value is significantly larger than the average cell size (≈ 0.8 mm) for pan bread (Zghal et al 1999, 2001), but it is consistent with the expected coarser crumb of French bread. The values (%d1) reported in Table II also show a significant negative effect of the sugar-rich fraction (F2.2) and a slightly positive effect of fraction F2.1, mainly composed of soluble proteins and pentosans. Variations of (%d1) values confirm the results obtained from ERDIL curves for defatted doughs. They underline the importance of defatted F1 to obtain a finer crumb because (%d1) is at least 56%, whereas defatting F2 only leads to 40%. The other values of gray level fraction are also modified according to the general trend of a reduction of larger gas cells for slices from dough containing def F1. Defatting and addition of PIN have similar effects on the distribution of gas cell fraction (Fig. 7). Continuous increase of (%d1) from 38% (CNS) to 44 and 51%, respectively, is observed when 0.1 and 0.2% PIN are added. This trend is enhanced with defatted flour (53%) and def CNS with 0.1% PIN, giving the finest crumb structure (%d1 58%). Although differences from one composition to another may not always be significant when considering limit size values of 2 mm and more, a striking feature is that the opposite trend to (%d1) is always observed in these cases (Fig. 7). Either for F2 decreasing or defatting and PIN adding, Figs. 6 and 7 show that the increase of (%d1) is concomitant to a narrower size distribution.

All these results show that (%d1) discriminates crumbs from various dough compositions. Characterizing French bread crumb is well adapted because 1 mm is the limit value for perception by the naked eye when bread scoring. Moreover, 1 mm is lower than the threshold value determined by the ERDIL technique. For these reasons, (%d1) may be considered as a crumb fineness indicator. It is consistent with the crumb fineness definition (number of cells per unit area) suggested by Zghal et al (1999). Zghal et al (2001) also found that crumb uniformity is directly correlated to fineness. Figures 6 and 7 also show that the larger the value of (%d1), the narrower the distribution of the gas cell size and, accordingly, the more uniform the crumb cellular structure. Hence, by symmetry (%d1) also provides information on crumb heterogeneity, which is a criterion for French bread score. When plotting values of specific volume obtained for all doughs against those measured

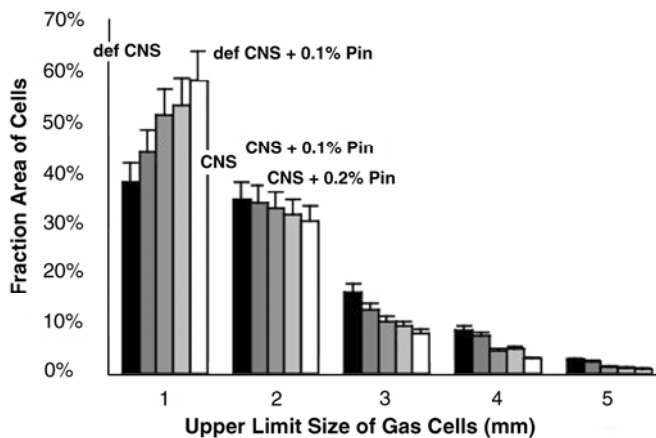


Fig. 7. Distribution of gas cells area fraction for different reconstituted doughs (left to right) defatted standard flour, addition of 0.1% puroindolines to defatted flour, commercial bread flour (CNS), addition of 0.1 and 0.2% puroindolines,

by digital image analysis for this fineness indicator, an overview of the effect of all the modifications of dough composition on bread volume and cell structure can be represented (Fig. 8). Clearly, soluble fraction modifies both volume and fineness concomitantly because good correlation is obtained between these two features ($r^2 \approx 0.9$) when only F2 addition to F1 is considered. Conversely, PIN addition and defatting modify fineness without significant effect on loaf volume in the concentration range tested.

Bread Volume, Crumb Texture, and Flour Composition

Both volume and texture characteristics strongly depend on how gas cells expand in the dough, merge or not, and stabilize all along the breadmaking process. Cells expansion depends on the ability of cell walls to retain gas but is also limited by dough bulk viscosity. All these phenomena may be influenced in a different manner by the different components of the fractions modified here. The larger the incorporation of soluble fraction (F2), the larger the specific volume and the coarser the cellular structure, or grain crumb because (%d1) decreases. Soluble fraction F2 is a motor for expansion because sugars provide the carbon source for the production of CO_2 by yeast cells. It could also act as a gas cell stabilizer by modifying dough bulk rheological properties. Soluble proteins and pentosans participate in the stabilization of the expansion of gas cells during proofing and baking (Gan et al 1995). Fraction F2.1 contains water-extractable pentosans that are known for improving loaf volume (Courtin et al 1999). Meanwhile, increasing content of fraction F2, F2.1, or F2.2 increases the foaming capacity and foaming stability to a plateau, indicating that interfaces are saturated with surface active components (results not shown). The importance of foaming properties of soluble components in dough microstructure and gas retention, responsible for final crumb fineness, has also been discussed by Gan et al (1995). This is in agreement with the increase of crumb fineness with addition of F2.1 observed in our study. Conversely, the decrease of fineness with added F2.2 would underline the antagonistic roles of sugars: gas production promoter and bubble growth reducer.

Figure 8 also shows that the addition of puroindolines or the use of defatted flour or corresponding fractions leads to a finer texture, that is that it increases the area fraction of gas cells <1 mm in size, whereas the specific volume remains rather constant, within experimental error interval. These results are in fair agreement with previous results that have shown that defatted and nondefatted flour could give rise to bread with identical volumes (MacRitchie and Gras 1973). The fact that cellular structure becomes finer for breads made from defatted CNS and from defatted F1 is

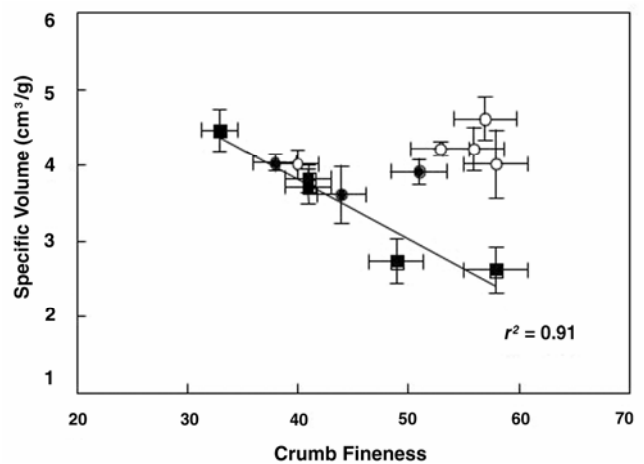


Fig. 8. Specific volume (cm^3/g) of breads from different reconstituted doughs as a function of crumb fineness, defined by the area fraction of gas cells <1 mm (%) (■ addition of soluble F2 to insoluble fraction F1; ● modification of standard flour; ○ addition of puroindolines and defatting. Regression is performed on ■ points: F2 added to F1 only).

in agreement with results that show that the extraction of lipids leads to finer crumb grain (MacRitchie 1981); but they seem in contradiction with the commonly accepted contribution to gas cell stability by lipids covering part of its surface (Keller et al 1997). This may be due to the fact that no effect of a specific fraction of lipids on texture is described. Indeed, removal of lipids modifies tension surface properties on the liquid layer around the gas cell (Keller et al 1997). But lipids present in soluble fraction (F2) have no effect on foam properties and texture.

Whatever the role of lipids and their chemical nature, bread-making is possible in the absence of lipids (MacRitchie 1981) because other surface active constituents participate in the stabilization of the interfacial film. This is illustrated by the addition of puroindolines to a 0.1% endogenous PIN content flour, defatted or native, which strongly modifies crumb cellular structure because (%d1) increases (Fig. 8). Moreover, the addition of 0.2% PIN to native flour leads to the same crumb grain as the bread from defatted flour. These results may be interpreted in relation with the influence of both components, puroindolines and lipids, on foaming properties, which depend on surface tension. Surface tension results from the competition between the proteins and lipids. In line with this interpretation, Dubreil et al (1998) suggest that the puroindoline-to-lipids ratio could be, with the nonpolar-to-polar lipids ratio, the most relevant biochemical parameter for predicting the structure of bread crumb (McCormack et al 1991). The repartition of lipids at the gas cell surface is affected by the ability of puroindolines to form strong lipid-protein complexes (Douliez et al 2000). Indeed, puroindolines prevent adsorption of wheat lipids to air-water interfaces, opening the possibility for other surface active components, especially soluble proteins, to form a stable film around gas bubbles. By localization of PIN-a and lipids in unyeasted dough, Dubreil et al (2002) have shown that puroindolines have a detergent-defatting effect preventing gas bubbles from coalescing and film rupture by lipid aggregates. Therefore, addition of puroindolines led to a homogeneous size distribution of gas cells in wheat dough, similar to that observed for solvent-defatted wheat flour. Conversely, the adsorption of lipids into the film layer promotes coalescence, finally leading to a more heterogeneous crumb.

These hypotheses attempt to link observations made on baked yeasted dough with those performed on mixed unyeasted doughs. They raise significant uncertainties by discarding modifications occurring during baking. Therefore, they require further experiments on bubble size after fermentation before they can be confirmed.

CONCLUSIONS

In agreement with our starting purpose, this study has allowed us to set up a procedure of image analysis using morphological treatment that avoided any critical step of thresholding for segmentation. Besides data on cell wall thickness and classification possibilities gained by this method, distribution size of gas cells fraction were obtained and, from those, an indicator was defined for the fineness of crumb cellular structure: the gray level fraction of cell sizes <1 mm. A consequence is that heterogeneity may be ascertained and this is of importance for French bread because an heterogeneous aspect of crumb is often appreciated by the consumer for this product. Such a conclusion could be obtained by performing breadmaking tests with fractionated-reconstituted doughs with different compositions of lipids, soluble proteins, sugars, and nonstarch carbohydrates (pentosans) that provide a large set of variation in specific volumes and crumb cellular structures.

In addition to the important role of gluten proteins in forming the correct network to obtain leavened products, the important role of many minor flour components in breadmaking is confirmed. The importance of the soluble fraction of wheat flour is stressed because it affects both loaf volume and crumb fineness in an

opposite way. Conversely, the role of puroindoline-lipid balance for increasing fineness is underlined. The full interpretation of the role of every component is not possible at this time because fractions are enriched in such components, but not purely composed, and interactions should not be discarded. However, it can be suggested that sugars increase gas production and decrease dough viscosity, while solubles, proteins, and pentosans stabilize gas cells during expansion in relation to the detergent-defatting role of puroindolines. These trends need confirmation at least in two directions: gas cell distribution in dough at the end of fermentation and rheological properties of dough reconstituted or not, using the same fractions.

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