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Chemometric Approach to Characterization of Flour Mill Streams: Chemical and Rheological Properties

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Abstract The aim of the present study was to utilize chemometric methods (the principal component analysis and hierarchical cluster analysis) for monitoring the certain aspects of flour mill streams quality and their interrelation to selected rheological properties. Thirty-seven flour mill streams were separated from industrial mill of 300 t/day capacity. All flour streams were analyzed for ash, protein, wet gluten, and damaged starch content and rheological properties as determined by Brabender Farinograph, Extensograph, and Amylograph. The obtained results indicated that break, sizing, and reduction flour streams exhibited different rheological behavior in relation to a change in protein, wet gluten, ash, and mechanically damaged starch content within the milling passages. Rheological properties of dough during mixing and kneading as well as during extension were different with regard to the technological phase of milling from which they were extracted. The obtained results could be utilized for selection of certain flour streams in production of special-purpose flours.

Keywords Flour mill streams · Chemical properties · Rheological properties · Principal component analysis

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Introduction

The main objective of industrial flour milling is to extract the maximum quantity of flour of desired quality. It is accomplished by the successive grinding and sieving operations during which the gradual fragmentation of wheat kernel occurs. Industrial wheat milling process comprises three main stages: grinding within roller mills, sieving, and purifying. The grinding stage comprises several phases—passages where certain amount of flour is produced, removed from the process and combined to provide final flour (Pomeranz 1988; Kent and Evers 1994; Posner and Hibbs 1997; Owens 2001; Sugden and Osborne 2001). A comprehensive knowledge about the distribution of certain chemical, biochemical, and rheological properties between mill streams is an important issue for optimization of final product quality (Ramseyer et al. 2011; Liu et al. 2011). Therefore, the determination of different aspects of quality of flour mill streams has been the focus of attention of numerous authors—the content and distribution of total protein and ash (Prabhasankar et al. 2000; Loza-Garay and Flores 2003; Okrajková et al. 2007), wet gluten (Pojić et al. 2004), lipids and fatty acids (Prabhasankar and Haridas Rao 1999; Prabhasankar et al. 2000), enzymes (Rani et al. 2001; Gebruers et al. 2002; Every et al. 2006a), damaged starch (Sutton and Simmons 2006; Banu et al. 2010; Pojić et al. 2012), pentosans (Wang et al. 2006a; Ramseyer et al. 2011), and antioxidants (Engelsen and Hansen 2009). Protein composition of different flour mill streams has also been investigated (Menkovska et al. 2002; Sutton and Simmons 2006; Wang et al. 2006b; Okrajková et al. 2007; Liu et al. 2011) as well as distribution of sulfur and free amino acids (Liu et al. 2011). The diversity in protein composition of

flour mill streams as well as the presence of variable amount of certain kernel constituents influences the different rheological properties of flour mill streams (Pojić et al. 2004; Banu et al. 2010; Liu et al. 2011). The data on the distribution of certain constituents and functional properties between flour mill streams could be utilized for the monitoring of the efficiency of milling procedure, mill settings, and blending of flour mill streams to obtain certain end-use quality that meet specific customer demands (Prabhasankar et al. 2000; Spasojević et al. 2000; Liu et al. 2011).

The implementation of more efficient tools for monitoring, optimization, characterization, handling of raw materials, intermediate and final products, as well as for prediction of quality throughout the whole production chain has become a must for the modern food industry if it strives for competitive position in the market. Throughout the production process, a number of different measurements are performed resulting in a large amount of data, used for one specific purpose (Bro et al. 2002). However, by combining all available information, the extraction of even more relevant information from the collected data is possible by application of multivariate statistics, mathematical modeling, and computing—all of them combined into a highly multidisciplinary research discipline—chemometrics (Bro et al. 2002; Gemperline 2006). It has become an irreplaceable tool for exploratory analysis of large datasets, multivariate quality assurance and quality control, detection of adulteration, estimation of chemical, physical, and sensory properties of foods (Gemperline 2006). Despite the wide application in food science, relevant literature is lacking in examples related to the use of chemometrics in studying the quality characteristics of flour mill streams, with the exception of the study by Dornez et al. (2006) who utilized the principal component analysis to study the distribution of arabinoxylans, endoxylanases, and endoxylanase inhibitors in industrial wheat roller mill streams. The application examples of chemometrics in the quality control of processed food with spectroscopic data are numerous, and beyond the scope of this study. Another example on the application of chemometrics in the quality control of processed food involved the utilization of chemometrics to study the influence of commercial bread improvers on dough rheology and hearth bread properties (Aamodt et al. 2003), the monitoring of authenticity of Brazilian UHT milk (Souza et al. 2011), the characterization of Brazilian lager and brown ale beers (Granato et al. 2011), and the discrimination between low- and full-fat yogurts (Cruz et al. 2013).

The objective of this study was to characterize the flour mill streams in terms of their chemical and rheological properties and to assess the interdependence between them by using two chemometric techniques—principal component analysis (PCA) and hierarchical cluster analysis (HCA).

Materials and Methods

Samples

Thirty-seven wheat flour mill streams were obtained from a commercial wheat flour mill (capacity 300 t/day): 8 break mill streams (1B-I_1, 1B-I_2, 2B-I_1, 2B-I_2, 3B-I_2, 3B-I_2, 4B-I_1, and 4B-I_2), 11 sizing mill streams (1R-I_1, 1R-I_2, 1R-II_1, 1R-II_2, 1R-III_1, 1R-III_2, 2R-I_1, 2R-I_2, 2R-II_1, 2R-II_2, and 2R-III), 17 reduction mill streams (1M-I, 1M-II, 2M-I, 2M-II, 3M-I, 3M-II, 3M-III, 4M-I, 4M-II, 5M-I, 5M-II, 6M, 7M, 8M, 9M-I, 9M-II, and 9M-III) and one bran duster flour stream (Fs–Vs). The flour stream samples were collected in accordance with the milling diagram during a fixed time interval taking into account the succession of the milling passages (Fig. 1).

Methods

The selected physicochemical properties of flour mill streams were determined as follows: moisture content according to ICC 109/1, protein content according to ICC 105/2, ash content according to ICC 104/1, and wet gluten content according to ICC 106/2 (ICC 1996) and damaged starch content according to AACC method 76–33.01 (AACC 2000). Selected rheological properties included Brabender Farinograph water absorption and softening degree determined according to ICC 115/1; Brabender Extensograph energy, dough resistance, and extensibility according to ICC 114/1; and Brabender Amylograph peak viscosity ICC 126/1. All selected properties of flour streams were analyzed in duplicate.

Data Analysis

A one-way analysis of variance (ANOVA) was used to test the significant differences in chemical and rheological quality between flour mill streams. ANOVA was followed by Fisher's least significant difference test, where the differences between means at the 5 % level ($p<0.05$) were considered significant.

The subsequent data analysis included two pattern recognition methods, the PCA and HCA applied to the data for each technological phase of milling—break, sizing, and reduction. Therefore, three different matrices were formed: 8 rows (samples)×11 columns (quality indicators) for break phase, 11 rows (samples)×11 columns (quality indicators) for sizing phase, and 18 rows (samples)×11 columns (quality indicators) for reduction phase (Cruz et al. 2013). PCA and Pearson correlation coefficients (r) were used for finding the relationships between selected quality properties of flour mill streams. PCA was performed following the mean centering, performed as a preprocessing method to equalize

Q1

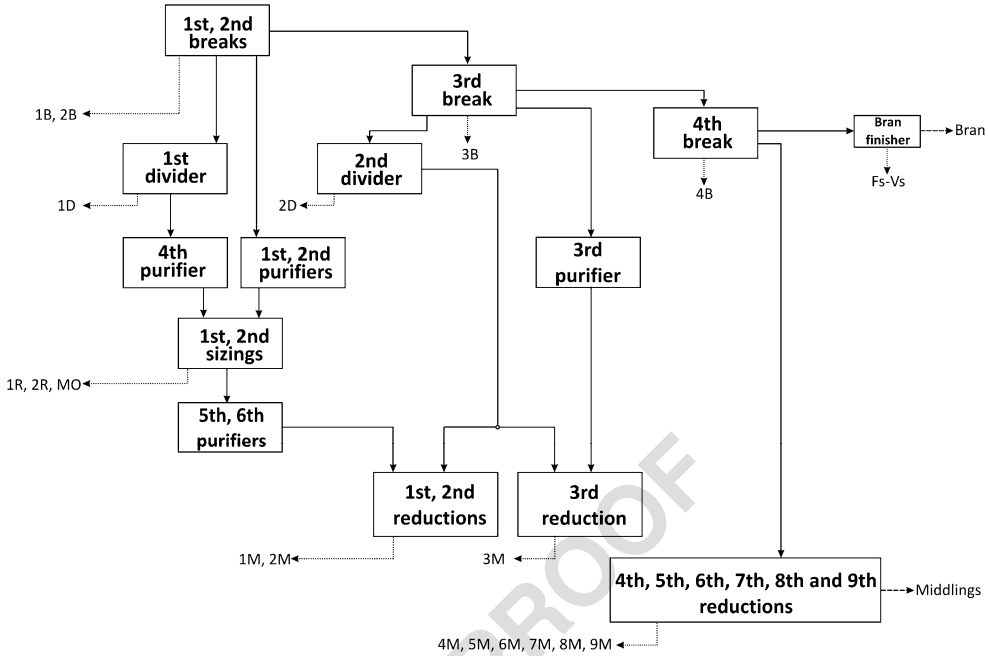

Fig. 1 A simplified mill flow diagram

the statistical importance of all the variables, with cross-validation (Beebe et al. 1998; Aamodt et al. 2003; Granato et al. 2011). HCA was performed following autoscaling as a preprocessing step. Sample similarities were calculated on the basis of the squared Euclidean distance, while the Ward hierarchical agglomerative method was used to establish clusters (Granato et al. 2011; Cruz et al. 2013). Statistical analysis was carried out using the software XLSTAT, version 2012.2.02 (Addinsoft, NY, USA).

Results and Discussion

Physicochemical Properties of Flour Mill Streams

Break Flour Mill Streams

The role of break system is to open the wheat kernel and to gradually scrape the endosperm from the bran throughout four break passages (1B, 2B, 3B, and 4B), which result in stock with maximally possible large middlings and minimal quantity of flour with minimal bran contamination. Certain amount of flour is produced within the break stage either due to releasing particles from fractured endosperm or particle attrition (Kent and Evers 1994; Posner and Hibbs 1997; Catterall and Cauvain 2007). The ground stock from the first two break passages are additionally classified on the two grading systems (D1 and D2), where additional amount of flour is separated (Fig. 1). This flour is not a result of grinding, but extended sieving of stock from the preceding breaking passages. Physicochemical properties of break flour streams indicated a wide variation in their quality (Table 1). Protein content (P) of break flours gradually increased over the successive break passages from 11.7 to 16.1 % dry matter (dm) being in accordance with the previously reported results (Prabhasankar et al. 2000; Rani et al. 2001; Dornez et al. 2006; Wang et al. 2007; Banu et al. 2010; Sakhare et al. 2012). The increase in protein content of break flours is affected by the increase in the presence of peripheral endosperm and bran particles rich in protein (Wang et al. 2007). Wet gluten content followed the similar trend as protein content and gradually increased over the successive break passages from 31.3 % for 1B to 39.7 % for 3B. Ash content (A) of break flours varied between 0.44 and 0.75 % dm with noticeable increase from 2B to 4B due to gradual roll gap decrease which resulted in release of aleurone layer, fine bran, and germ particles along with the endosperm particles (Sakhare and Inamdar 2011). Although 1B flour often has the lowest ash content among break flours, 1B flour was characterized by higher ash content in relation to 2B flour stream, probably due to the release of accumulated mineral dust from kernel crease (Sakhare and Inamdar 2011). Damaged starch content showed an increasing trend from 12.7 to 20.2 ~~Unité~~ Chopin Dubois (UCD) as the grinding progressed from 1B to 4B showing the opposite tendency observed by Banu et al. (2010). Q2

Rheological properties of dough as determined by empirical rheological tests such as Brabender Farinograph, and Extensograph reflect the processing quality of flours and are commonly used as a part of routine quality control within the baking industry (Stojceska et al. 2007; Dapčević Hadnađev et al. 2011). The presence of different anatomic parts of wheat kernel in break flours caused their different rheological behavior (Hayta and Schofield 2004).

Table 1 Selected physicochemical and rheological properties of break flour streams

Passage	P % dm	WG %	A % dm	WA %	SD BU	E cm^2	R BU	Ex mm	PV BU	DS UCD
1B-I_1	11.8±0.06 n	31.7±0.43 e,f	0.48±0.00 k	50.0±0.25 s	82±2.9 e,f,g	44±1.5 o,p	172±2.9 l,m	163±2.1 f,g	173±2.9 k	12.7±0.14 q,r
1B-I_2	11.7±0.05 n,o	31.3±0.20 f,g	0.49±0.01 j,k	50.5±0.15 r,s	73±2.9 f,g, h	48±1.5 o	174±5.1 l,m	169±2.5 e	180±5.0 k	13.2±0.21 q
2B-I_1	12.8±0.01 k,l	32.2±0.10 e	0.45±0.00 l	52.1±0.40 o,p,q	48±2.9 k,l,m	103±4.6 c,d	285±5.0 e	178±3.8 c,d	222±5.8 h,i	14.4±0.28 p
2B-I_2	12.7±0.02 l	31.9±0.38 e	0.44±0.01 l	52.2±0.47 o,p,q	53±2.9 j,k,l	99±4.0 d,e	292±2.9 d,e	182±3.8 c	237±2.9 h	16.0±0.21 n,o
3B-I_1	15.1±0.03 e	39.4±0.10 a,b	0.44±0.00 l	54.0±0.30 m,n	43±2.9 l,m,n	122±4.2 a	230±3.0 i,j	231±4.6 b	220±5.0 i	17.6±0.14 j,k
3B-I_2	15.3±0.09 e	39.7±0.76 a	0.57±0.01 i	54.2±0.45 m,n	50±5.0 k,l,m	126±5.0 a	232±3.6 i,j	235±2.5 b	217±2.9 i	17.2±0.14 k,l
4B-I_1	16.3±0.10 d	39.0±0.75 b,c	0.73±0.03 g	56.9±0.57 k,l	55±5.0 j,k,l	106±3.3 c	190±4.2 k	248±4.2 a	220±5.0 i	20.2±0.14 h,i
4B-I_2	16.1±0.02 d	38.6±0.23 c	0.75±0.01 g	56.6±0.35 l	60±2.9 i,j,k	112±5.5 b	193±4.7 k	251±2.1 a	225±5.0 h,i	20.2±0.14 h,i

Mean value±standard deviation. Figures followed by the same letter within the same column are not significantly different ($p<0.05$)

P protein content, *WG* wet gluten content, *A* ash content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *R* Extensograph resistance, *Ex* Extensograph extensibility, *PV* peak viscosity, *DS* damaged starch

PCA was performed for all analyzed chemical and rheological characteristics in order to observe the quality variation among the flour mill streams and their interrelationships (Martens and Martens 2001).

This method was chosen due to its suitability for identifying the patterns in data and data display which provides evident observation of their similarities and differences indicating the correlation between variables. The original variables are compressed into a small number of new variables—principal components (PCs) describing independent variation structures in the data. PCs are used as new axes in a plot of samples (score plot) and a corresponding plot of variables (loading plot). Hence, the properties that are related and those that are most important in distinguishing between samples are easily visualized. Two variables that appear close to each other in the plot indicate their positive correlation, whereas variables appearing on opposite sides are negatively correlated. Variables located in perpendicular directions along the PC axis are independent of each other (Aamodt et al. 2003; Dornez et al. 2006; Shin et al. 2010).

Figure 2a shows the PCA score plot of the first two principal components (PC1 vs. PC2) derived from selected physicochemical properties of break flours, revealing the similarities and differences between them. A clear separation of the flour samples from different passages as well as grouping of the flour samples originated from the same passage is noticeable (Fig. 2a). PCA loading plot revealed that 93.39 % of the variability in the data was explained by the first two PCs, where 67.40 % of the variability was explained by PC1 and 25.99 % by PC2 (Fig. 2b). By examination of PCA loading plot, it was noticeable that increase in protein, wet gluten, and mechanically damaged starch content affected the increase in the Farinograph water absorption (r=0.97, r=0.86, and r=0.93, respectively) which varied from 50.0 to 56.9 % as the grinding progressed from 1B to 4B (Fig. 2b, Table 1). The tail-end break flours appeared to have more acceptable water absorption values for bread making than those from head-end passages. The intensity of mechanical damage of starch influences the quality of flour in terms of water absorption, rheological behavior, and fermentation of the leavened products thus determining bread crumb firmness and crust color (Di Stasio et al. 2007). Higher water binding capacity is also attributed to the presence of arabinoxylans, mainly located in the aleurone and bran (Pomeranz 1988; Dornez et al. 2006; Wang et al. 2006a; Fustier et al. 2009a, b; Banu et al. 2010; Ramseyer et al. 2011). Ramseyer et al. (2011) reported strong correlation of total and water-unextractable arabinoxylan content with ash content, which reflected the presence of bran in flour streams suggesting the differences in flour functionality. Protein and wet gluten content were positively correlated with the Extensograph extensibility (r=0.99 and r=0.96, respectively) and energy (r=0.79 and r=0.77, respectively). The Extensograph

extensibility exhibited the gradual increase from 163 mm for 1B to 251 mm for 4B, while the Extensograph energy gradually increased from 44 cm^2 for 1B to 126 cm^2 for 3B (Table 1). The positive relationship between protein content and dough extensibility along the first PC for patent flour was previously reported by Aamodt et al. (2003). Fustier et al. (2009a, b) reported the positive relation between protein content and the Alveograph deformation energy, being the measure of extensional work as Extensograph energy is. Dobraszczyk and Salmanowicz (2008) reported close relation between protein content and Kieffer maximum extensibility and area under Kieffer force–distance curve. Kieffer dough extensibility rig measures large deformation of dough in uniaxial extension providing results that correspond to Extensograph results (Dunnewind et al. 2004). Resistance to extension of break flour streams was not related to measured chemical properties of break flours and manifested no particular trend over the break passages, being the lowest for 1B (170 BU) and highest for 2B (290 BU; Table 1). Although Aamodt et al. (2003) reported that resistance to extension was related to flour protein content along the first PC, it must be noted that the distribution of the various protein groups occurs during milling process, which is reflected in a corresponding variation of the rheological properties of flour streams (Wang et al. 2007). The obtained results were also consistent with the results of Dobraszczyk and Salmanowicz (2008) who reported no relation between protein content and Kieffer maximum force that corresponds to the Extensograph resistance to extension. The rheological properties of flour mill streams are also dependent on the presence of glutathione, being impaired with the increasing content of glutathione typical for the tail-end passages (Every et al. 2006b).

The peak viscosity of break flours as being located in perpendicular directions along the PC1 axis appeared to be independent of selected chemical parameters (Fig. 2b). The peak viscosity values varied within narrow range 175–225 BU, indicating that the viscosity was not a consequence of mechanical damage of starch but of flour origin.

HCA was performed, primarily due to its ability to reveal a natural groupings (or clusters) within a given data set, in the form of a special graph—dendrogram. Thus, the visualization of clusters and correlations among samples or variables is enabled (Souza et al. 2011). Similar to PCA, HCA revealed three distinct clusters of break flour mill streams (Fig. 2c). Cluster 1 contained the initial break flours (1B-I1 and 1B-I2), being in accordance with the sample grouping in the PCA score plot. The loading plot indicated that their position was related to the highest Farinograph softening degree. Cluster 2 contained flour streams from second break passage (2B-I1 and 2B-I2), remained separated from other samples indicating that their Extensograph resistance significantly differed from the other break flours. Cluster 3 contained the terminal break flour streams (3B-I1, 3B-I2, 4B-I1, and 4B-I2) which appeared separated from each other in PCA score plot due to significantly different values of the Extensograph energy and extensibility, ash, and damaged starch content (Fig. 2a and b, Table 1).

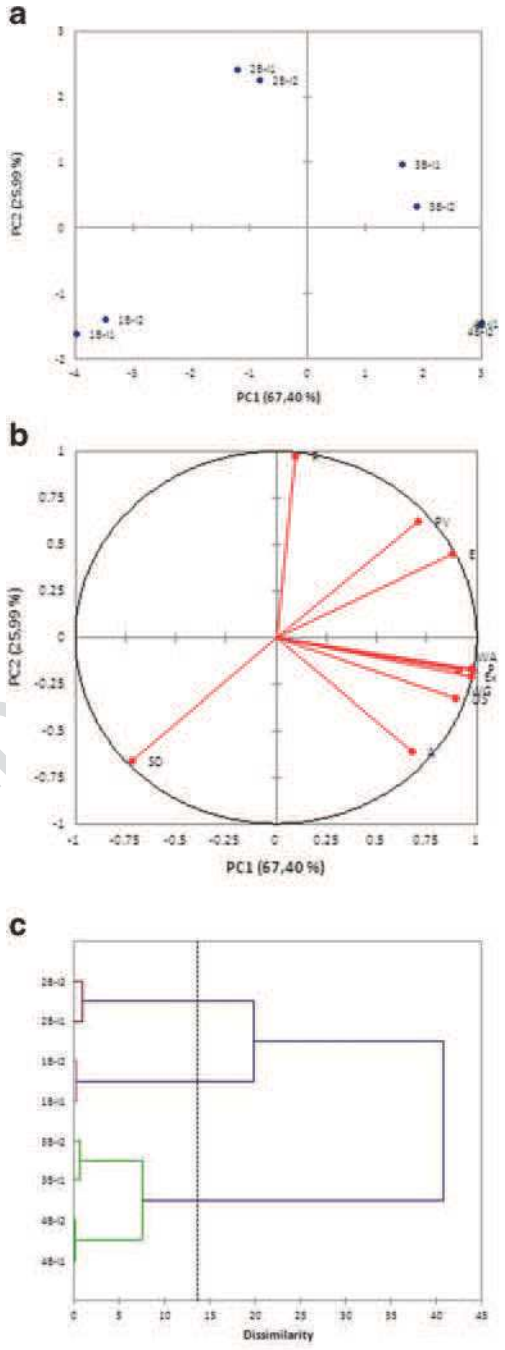

Fig. 2 Break flour mill streams: PCA score plot (PC1 vs PC2) (**a**). PCA loading plot (PC1 vs PC2) for physicochemical and rheological properties (**b**). HCA dendrogram (**c**). *P* Protein content, *A* ash content, *WG* wet gluten content, *DS* damaged starch content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *Ex* Extensograph extensibility, *R* Extensograph resistance to extention, *PV* amylograph peak viscosity

Sizing Flour Mill Streams

The main objective of the sizing system is to separate bran and endosperm particles that remained together after breaking passages, to reduce the size of large endosperm particles and to enhance the particle size uniformity. The selected physicochemical and functional properties of sizing flour mill streams are shown in Table 2. Due to the absence of bran and peripheral endosperm particles, sizing flours were of lower ash content compared to that of break flours. Ash content of sizing flours varied within narrow range of 0.30–0.34 % dm, indicating that flour originates from the central parts of endosperm. Moreover, the sizing flour streams were characterized by lower protein and wet gluten content in comparison to those of breaking passages, varying within the range of 10.4–11.8 and 23.0–33.0 % dm, respectively. Damaged starch content of sizing flours varied within broad range from 0.2 to 23.1 UCD, with no regular trend over the sizing passages.

Although no discernable pattern in variation of selected chemical and rheological properties of mill streams within the sizing system could be noticed, the PCA score plot of the first two principal components (PC1 vs. PC2) revealed clear separation of flours originated from different sizing passages. The PCA loading plot revealed that 73.48 % of the variability in the data was explained by the first two PCs, where 45.82 % of the variability was explained by PC1 and 27.66 % by PC2 (Fig. 3a). Farinograph water absorption increased with the increase in protein (r=0.75), ash (r=0.80), and damaged starch content (r=0.98; Fig. 3b). Moreover, increase in wet gluten content caused the decrease in Farinograph softening degree (r=−0.74), while Extensograph energy (r=0.62) and extensibility (r=0.67) increased with the increase in protein content (Fig. 3b). Increase in mechanically damaged starch granule content caused the decrease in Amylograph peak viscosity (r=−0.94) due to increased susceptibility of mechanically damaged starch granule to α-amylase hydrolysis.

Similar to PCA, HCA revealed three distinct clusters of sizing flour mill streams (Fig. 3c). Cluster 1 contained the first flours from the first two passages of sizing (1R-I1, 1R-I2, 2R-I1, and 2R-I2), which in PCA score plot appeared separated from each other due to significantly different values of Farinograph water absorption, protein, and damaged starch content (Fig. 3a and b, Table 2). Cluster 2 comprised the second and third flours from the initial passage of sizing (1R-II1, 1R-II2, 1R-III1, and 1R-III2), while cluster 3 comprised the remaining flours from the second sizing passage (2R-II1, 2R-II2, and 2R-III), which corresponded to the sample grouping as appeared in PCA score plot (Fig. 3a).

Reduction Flour Mill Streams

The role of the reduction system is to reduce the size of endosperm particles into as much high-quality flour as

Table 2 Selected physicochemical and rheological properties of sizing flour streams

Passage	P % dm	WG %	A % dm	WA %	SD BU	E_2 cm^2	R BU	Ex mm	PV BU	DS UCD
1R-I_1	11.8±0.01 n	29.6±0.10 i	0.33±0.01 n,o,p	56.2±0.57 l	23±2.9 p,q	101±3.2 c,d,e	327±2.9 c	156±4.7 h,i,j	288±2.9 e,f	23.1±0.14 f
1R-I_2	11.8±0.02 n	29.0±0.10 i	0.34±0.00 n,o	56.7±0.61 k,l	35±5.0 n,o,p	97±4.7 e	350±5.0 b	150±2.3 k,l	283±2.9 f	22.4±0.14 f,g
1R-II_1	11.5±0.06 o,p	31.1±0.30 g	0.32±0.00 o,p,q	53.5±0.25 m,n,o	23±0.0 p,q	104±5.1 cd	360±5.0 a,b	154±3.2 i,j,k	380±5.0 b	13.2±0.07 q
1R-II_2	11.3±0.04 p,q	29.1±0.35 i,j	0.32±0.00 o,p,q	53.0±0.41 n,o,p	18±2.9 q	90±3.2 f	365±5.0 a	139±3.2 m	380±5.0 b	12.2±0.28 r
1R-III_1	11.3±0.01 p,q	27.4±0.30 k,l,m	0.31±0.01 p,q	51.2±0.30 q,r,s	23±2.9 p,q	100±6.2 d,e	322±2.9 c	164±4.0 e,f	425±0.0 a	0.2±0.07 x
1R-III_2	11.0±0.02 r,s,t	33.3±0.20 d	0.30±0.01 q	51.1±0.55 q,r,s	23±2.9 p,q	84±2.5 g	302±2.9 d	150±1.5 k,l	428±2.9 a	2.0±0.07 w
2R-I_1	11.1±0.01 q,r,s	27.5±0.04 k,l,m	0.31±0.00 p,q	54.6±0.47 m	52±2.9 k,l,m	62±3.0 k,l,m	222±2.9 j	155±2.1 h,i,j,k	288±2.9 e,f	16.2±0.21 m,n
2R-I_2	11.0±0.04 r,s,t	27.0±0.31 m,n	0.32±0.00 o,p,q	54.4±0.35 m,n	45±0.0 l,m,n	70±2.0 i,j	253±2.9 g,h	156±2.5 h,i,j	302±2.9 d,e	18.3±0.14 j
2R-II_1	10.7±0.05 u,v,w	26.6±0.20 n,o	0.30±0.01 q	51.7±0.38 p,q,r	28±2.9 o,p,q	75±3.5 h,i	363±5.8 a,b	126±3.0 o,p	393±5.8 b	4.4±0.07 v
2R-II_2	10.6±0.02 v,w,x	27.1±0.33 l,m,n	0.31±0.00 p,q	51.7±0.36 p,q,r	42±2.9 m,n,o	85±2.3 f,g	367±15.3 a	139±1.5 m	390±5.0 b	6.4±0.21 t
2R-III	10.4±0.01 x	23.0±0.39 s,t	0.32±0.01 o,p,q	51.5±0.55 q,r	65±0.0 h,i,j	80±2.5 g,h	370±5.8 a	131±3.2 n,o	385±5.0 b	5.5±0.07 u

Mean value±standard deviation. Figures followed by the same letter within the same column are not significantly different (p<0.05)

P protein content, *WG* wet gluten content, *A* ash content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *R* Extensograph resistance, *Ex* Extensograph extensibility, *PV* peak viscosity, *DS* damaged starch

possible. At this stage, bran and germ particles are flattened which enable their separation during sieving. The reduction flour mill streams were characterized by general gradual increase in protein (from 10.5 to 17.8 % dm) and ash (from 0.35 to 2.52 % dm) content being the highest in the tail-end reduction flours due to the increasing contamination of millstreams with aleurone, bran, and germ particles (Table 3). Prabhasankar et al. (2000) and Dornez et al. (2006) observed no regular trend in protein content increase from head-end to tail-end reduction passages, whereas Gebruers et al. (2002) indicated a regular trend in protein content increase for both break and reduction rolls. Dornez et al. (2006) indicated that the increase in protein content over reduction stage is not always obvious due to smaller differences in protein content of reduction passages in relation to that of break passages. Wet gluten content gradually increased from 18.0 % for 1 M to 30.5 % for 3 M. The content of mechanically damaged starch increased from 9.7 to 29.6 UDC being the highest in the tail-end reduction flours due to more severe grinding at tail-end reduction passages (Table 3). By studying the microstructure of tail-end flour streams from roller mill, Gangadharappa et al. (2008) indicated the presence of deformed A-type (lenticular shaped) and intact B-type (spherical shaped) starch granules in the very tail-end flour stream, whereas preceding flour stream contained slightly visible structural deformations of A type starch granules.

The qualitative differences within the reduction flours could be visualized in the PCA score plot, where tail-end reduction flours (6M–9M) were located to the left and separated from head-end reduction flours (1M–5M) to the right in the score plot (Fig. 4a). Bran duster flour stream (Fs–Vs) appeared to be entirely distinct from tail-end reduction flours. In the corresponding loading plot (Fig. 4b), 79.55 % of the variability in the data was explained by the first two PCs, where 62.51 % of the variability was explained by PC1 and 17.05 % by PC2. The increasing content of peripheral endosperm in flour resulted in increasing water absorption throughout the reduction system due to their higher water-binding capacity (Banu et al. 2010). In particular, the increase in water absorption was affected by the increase in protein (r=0.63), ash (r=0.58), and damaged starch content (r=0.49). The increase in Farinograph softening degree was affected by increase in protein (r=0.70), ash (r=0.66), and damaged starch content (r=0.54), showing the opposite trend in comparison to the break millstreams. Moreover, the increased presence of peripheral kernel particles rich in protein and minerals affected the decrease in Extensograph energy (r=−0.75 and r=−0.76, respectively) and resistance to extension (r=−0.84 and r=−0.74, respectively) being in contradiction with break flour mill streams. The opposite functional behavior between break and reduction streams in terms of total flour protein content was also observed by Wang et al. (2007) who reported the positive correlation between loaf volume and protein content of break streams, and negative correlation between loaf volume and protein content of reduction streams. The reason for diverse relation of certain

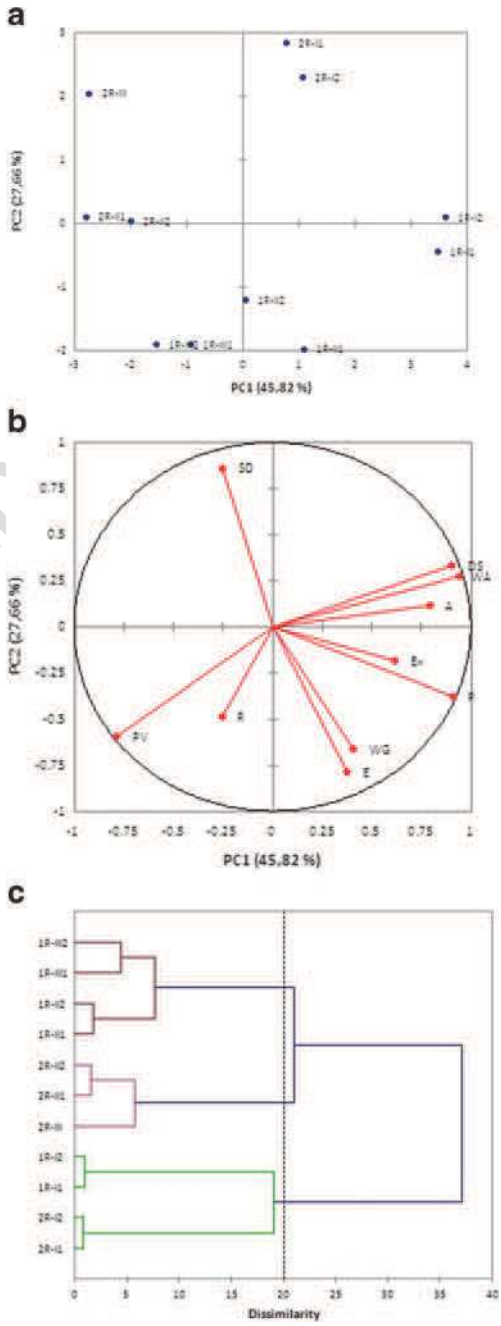

Fig. 3 Sizing flour mill streams: PCA score plot (PC1 vs PC2) (**a**). PCA loading plot (PC1 vs PC2) for physicochemical and rheological (**b**). HCA dendrogram (**c**). *P* Protein content, *A* ash content, *WG* wet gluten content, *DS* damaged starch content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *Ex* Extensograph extensibility, *R* Extensograph resistance to extention, *PV* amylograph peak viscosity

t3.1 **Table 3** Selected physicochemical and rheological properties of reduction flour streams

t3.2 t3.3 Passage	P % dm	WG %	A % dm	WA %	SD BU	E cm^2	R BU	Ex mm
t3.4 1M-I	10.8±0.12 t,u,v	23.1±0.34 s,t	0.35±0.01 n	58.1±0.15 j,k	63±2.9 h,i,j	66±3.2 j,k	302±2.9 d	131±2.0
t3.5 1M-II	10.5±0.01 w,x	22.7±0.02 t,u	0.38±0.02 m	58.5±0.21 j	78±2.9 e,f,g	57±3.5 m,n	303±2.9 d	124±2.5
t3.6 2M-I	11.2±0.03 q r	25.4±0.32 p	0.38±0.00 m	60.7±0.41 h,i	90±0.0 d,e	54±0.6 n	273±15.3 f	130±3.8
t3.7 2M-II	10.9±0.03 s,t,u	24.1±0.24 q	0.38±0.00 m	59.0±0.35 j	75±5.0 f,g,h	59±0.2 l,m,n	290±10.0 d,e	127±2.1
t3.8 3M-I	11.8±0.06 n	27.6±0.12 k,l	0.40±0.00 m	62.5±0.32 f,g	70±0.0 g,h,i	71±0.5 i,j	252±10.4 g,h	152±1.5
t3.9 3M-II	11.5±0.05 o,p	28.9±0.16 j	0.45±0.01 l	62.7±0.25 f,g	62±2.9 i,j,k	69±0.9 j	333±11.5 c	130±3.0
t3.10 3M-III	11.0±0.08 l	22.2±0.19 u	0.50±0.01 j,k	65.0±0.32 d,e	78±2.9 e,f,g	44±1.5 o,p	257±10.4 g	118±2.5
t3.11 4M-I	13.3±0.02 r,s,t	26.3±0.23 o	0.51±0.01 j	62.0±0.23 g,h	68±2.9 g,h,i	63±2.1 k,l	220±5.0 j	160±2.5
t3.12 4M-II	13.1±0.11 i,j	26.4±0.42 o	0.49±0.01 j,k	59.5±0.41 i,j	73±2.9 f,g,h	66±1.2 j,k	240±5.0 h,i	158±2.5
t3.13 5M-I	12.2±0.05 m	23.5±0.18 r,s	0.48±0.02 k	63.6±0.31 e,f	85±5.0 e,f	56±3.0 n	252±5.8 g,h	134±3.6
t3.14 5M-II	13.0±0.07 j,k	23.5±0.10 r,s	0.63±0.01 h	65.6±0.15 d	100±5.0 d	39±1.5 p	217±15.3 j	122±2.3
t3.15 6M	14.3±0.04 g	23.9±0.32 q,r	0.89±0.01 e	69.0±0.35 c	118±2.9 c	26±2.1 q	163±11.5 m	117±2.1
t3.16 7M	16.1±.03 d	27.5±0.26 k,l,m	1.35±0.01 d	68.6±0.15 c	123±2.9 b,c	23±3.0 q	112±2.9 n	148±2.1
t3.17 8 M	14.8±0.04 f	27.8±0.52 k	1.39±0.01 c	70.5±0.25 b	132±2.9 a,b,c	16±0.6 r	123±11.5 n	90±1.5 r
t3.18 9 M-I	17.8±0.05 a	18.0±0.50 w	2.52±0.02 a	72.0±0.29 a	130±2.9 a,b,c	n/a	n/a	n/a
t3.19 9 M-II	17.3±0.05 b	19.1±0.60 v	2.09±0.01 b	71.5±0.25 a,b	140±5.0 a	n/a	n/a	n/a
t3.20 9 M-III	16.7±0.12 c	18.5±0.62 w	2.07±0.01 b	58.8±0.25 j	80±0.0 e,f,g	n/a	n/a	n/a
t3.21 Fs-Vs	13.4±0.03 h	30.5±0.30 h	0.79±0.01 f	71.0±0.49 a,b	135±5.0 a,b	56±1.5 n	183±5.8 k,l	175±2.6

Mean value±standard deviation. Figures followed by the same letter within the same column are not significantly different ($p<0.05$)

P protein content, *WG* wet gluten content, *A* ash content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *R* extensibility, *PV* peak viscosity, *DS* damaged starch

rheological properties of dough of break and reduction millstreams might be associated with different distribution of protein fractions among them (Figs. 2b and 4b; Liu et al. 2011). Namely, the different rheological behavior of break and reduction flour streams could be explained by higher ratio of polymeric to monomeric proteins of break than of reduction streams (Wang et al. 2007). Break passages especially head-end passages release relatively pure endosperm particles, while tail-end milling passages tend to scrape off residual endosperm particles from peripheral layer of kernel, together with fine bran and germ particles (Sakhare et al. 2012). Resistance to extension was negatively correlated with damaged starch content. Jovanovich et al. (2003) indicated that the positive relation between Alveograph dough tenacity and damaged starch content regardless of the differences in wheat and milling fractions. When performing Alveograph test, which is conducted with constant moisture content of dough, flour containing higher damaged starch content would not be completely hydrated resulting in higher dough tenacity. The increased presence of peripheral kernel particles rich in protein, minerals, and amylolytic enzymes affected the decrease in Amylograph peak viscosity (r=−0.79 and r=−0.78, respectively). The fact that α-amylase is mostly located in the peripheral parts of a wheat kernel indicated similar distribution of α-amylase and ash content among flour mill streams, where tail-end flours were characterized by higher α-amylase activity (Rani et al. 2001; Every et al. 2002; Dornez et al. 2006). More intense shear stresses imposed on starch granules during reduction stage induced the stronger mechanical damage of starch granules than during break stage. Since the recorded peak viscosity is an indirect measure of the present α-amylase status along with the starch granule quality, the higher α-amylase activity and higher quantity of mechanically damaged starch in the tail-end reduction passages resulted in lower peak viscosity (Fustier et al. 2009a, b).

HCA revealed three distinct clusters of reduction flour mill streams (Fig. 4c). Cluster 1 contained the flour streams from head-end reduction passages (1M-I, 1M-II, 2M-I, 2M-II, 3M-I, 3M-II, 3M-III, 4M-I, 4M-II, and 5M-I) located to the left side in the corresponding PCA score plot due to the differences in Alveograph peak viscosity, Extensograph resistance and energy between this group and the remaining group of reduction flours. Cluster 2 included 5M-II, 6M, 7M, 8M, and Fs–Vs flours, while cluster 3 comprised the terminal reduction flour streams (9M-I, 9M-II, and 9M-III). The corresponding loading plot indicated that the position of these terminal reduction flours was related to their high protein and ash content (Fig. 4a and b).

The noticeable differences in the rheological behavior of break, sizing, and reduction flours could be generally attributed to the presence of different proportion of various anatomic part of a wheat kernel in them (Wang et al. 2007; Fustier et al. 2009a, b). In particular, flour streams differ in protein concentration and its molecular composition—amount and size distribution of polymeric and monomeric proteins, amount of free thiol groups, and amount of disulfide linkings (Menkovska et al. 2002; Sutton and Simmons 2006; Okrajková et al. 2007; Wang et al. 2007). Moreover, the

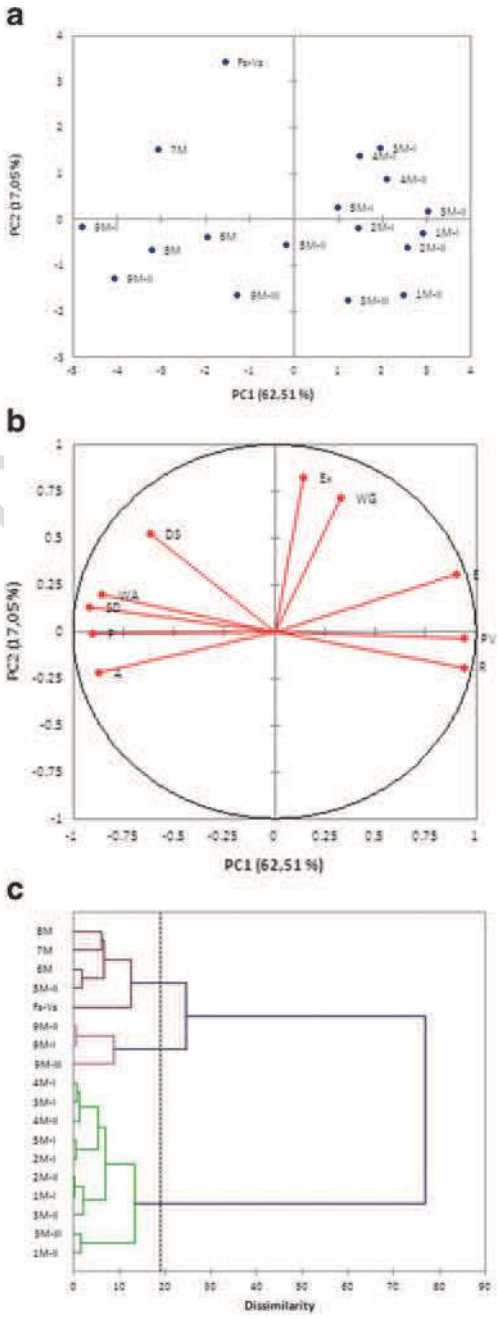

Fig. 4 Reduction flour mill streams: PCA score plot (PC1 vs PC2) (**a**). PCA loading plot (PC1 vs PC2) for physicochemical and rheological properties (**b**). HCA dendrogram (**c**). *P* Protein content, *A* ash content, *WG* wet gluten content, *DS* damaged starch content, *WA* Farinograph water absorption, *SD* Farinograph softening degree, *E* Extensograph energy, *Ex* Extensograph extensibility, *R* Extensograph resistance to extention, *PV* amylograph peak viscosity

variable distribution of lipids, fatty acids, enzymes (α-amylase, protease, lipoxygenase, polyphenol oxidase, peroxidase), and mechanically damaged starch granules within the flour mill streams also contribute to the variable rheological behavior of flour mill streams (Prabhasankar and Rao 1999; Prabhasankar et al. 2000; Rani et al. 2001; Di Stasio et al. 2007). The different rheological behavior of flour streams could be associated to different sulfur content between break and reduction flour streams, being the higher for break flours as indicated by Liu et al. (2011). Higher sulfur content is associated with the increase in the proportion of LMW subunits of glutenin and increased dough extensibility and decreased dough elasticity thereof (Zhao et al. 1999; Liu et al. 2011). Liu et al. (2011) indicated that sulfur content of flour mill streams supplemented with ash content appeared to be useful to evaluate their quality due to significant correlations with bread loaf volume, Farinograph peak time, and Extensograph parameters. Better rheological properties and higher gluten strength of break flours in comparison to those of reduction flours could be explained by lower degree of disulfide cross-linkings and higher amount of albumins and globulins mainly located in the peripheral parts of wheat kernel (Okrajková et al. 2007; Wang et al. 2007). Every et al. (2006a) reported higher concentration of enzyme peroxidase in the break flours, which oxidizes and cross-links ferulic acid residues on arabinoxylan and produces a secondary pentosan network through the gluten network that improves rheological properties of dough made from break flours. More apparent variability in rheological properties of break flours in comparison to those of reduction flour could be attributed to the amount of gliadin, sodium dodecyl sulfate (SDS)-soluble glutenin, and SDS-insoluble glutenin that changed markedly within the breaking stage in contrast to reduction stage (Sutton and Simmons 2006). Sutton and Simmons (2006) also found that tailing passages exposed to more intensive grinding and consequently more intensive molecular disruption, characterized by higher thiol content (Sutton and Simmons 2006) and higher damaged starch content (Jovanovich et al. 2003; Gangadharappa et al. 2008) affecting certain rheological and processing quality properties.

Conclusion

Flour mill streams from consecutive milling passages largely differ in composition, rheological properties, and hence in overall technological functionality. The selected chemometric techniques, the principal component analysis, and the hierarchical cluster analysis could be effectively used for the visualizations of the performance of milling procedure by monitoring the different aspects of flour mill streams quality and their interdependency. Break, sizing, and reduction flour streams exhibited different rheological behavior in relation to a change in protein, wet gluten, ash, and mechanically damaged starch content within the milling process. Rheological properties of dough made of flour streams recorded during mixing and kneading were different with regard to the technological phase of milling from which they were extracted. Softening degree of break flours was not related to measured chemical properties, while that of sizing and reduction flours was negatively correlated with wet gluten content and positively correlated with protein, ash, and damaged starch content, respectively. Moreover, the extensional properties of dough made of flour streams were dependent on the phase of milling from which they were extracted so that extensibility and energy was positively correlated with protein and wet gluten content of break and sizing flours. Energy and resistance to extension of dough made from reduction flours was negatively correlated with protein and ash content. Pasting properties of sizing and reduction flour streams were negatively correlated with ash and mechanically damaged starch content, while such dependence was not observed for break flours.

Although the results in this study were obtained by milling certain wheat within one milling diagram, it might be expected that the interrelation between certain aspects of flour millstream quality could be also applicable in the processing of wheat of different quality and/or using different milling diagram. Hence, the selection of certain flour streams for production of special-purpose flours, as well as the selection of appropriate flours for specific bakery products could be enabled.

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