Fortification of whole wheat flour with different iron compounds: effect on quality parameters and stability

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Abstract The aim of this study was to evaluate the effects of fortification of whole wheat flour with different iron compounds, such as ferrous sulfate (FS), ferrous fumarate (FF), reduced iron (RI), ferric sodium ethylenediaminetetraacetate (NaFeEDTA), microencapsulated ferrous sulfate (FSm) and microencapsulated ferrous fumarate (FFm), on quality parameters: color, titratable acidity, peroxide value (PV) and hexanal values, during 120 days of storage. An iron content of 1.38 mg/100 g was quantified in non-fortified whole wheat flour and after fortification, the iron levels ranged from 4.80 to 6.29 mg/100 g. The fortification of whole wheat flour with different iron compounds showed changes on the quality parameters evaluated during storage with exception of the color. The whole flour acidity was affected mainly by NaFeEDTA. Compounds FS and FFm presented the highest PV in whole flour after 30 days of storage. Whole flours fortified with FS and FSm presented higher hexanal levels after 30 and 90 days of storage, respectively. Whole flours fortified with RI and NaFeEDTA presented more stability on quality parameters evaluated during storage period. Therefore, the different iron compounds, when used on whole wheat flour fortification, affect differently the quality of the product during storage.

Keywords Quality · Degradation · Iron · Gas chromatography · Hexanal

Introduction

Wheat flour contain in its composition carbohydrates, water, protein and lipids in addition to vitamins and minerals. The lipid fraction is made up of approximately 51% of non-polar or neutral lipids and 49% of polar lipids and its stability may be affected by the composition of fatty acids (Cramer et al. 2005). Variations in the amount and composition of the whole wheat flour constituents may occur depending on the different proportions of bran and/or germ that may be reincorporated into the refined wheat flour (Gutkoski et al. 2008; Morita et al. 2002; Rutz 2012).

In the Brazilian Legislation the minimum proportion of bran and/or germ that may be reincorporated into the refined wheat flour for making whole wheat flour is not established (Brasil 2005). According to Pomeranz et al. (1977), the addition of approximately 7% of wheat bran into the refined flour does not cause significant changes in rheological and technologies properties.

Currently wheat flour must be fortified with iron in various countries to reduce the prevalence of iron deficiency anemia caused by the reduced consumption of bioavailable iron (Brasil 2002; WHO 2006). In Brazil, the Legislation allows the addition of different iron compounds: dehydrated ferrous sulphate (dry), ferrous fumarate, reduced iron, ferric sodium ethylenediaminetetraacetate (NaFeEDTA), and other compounds with bioavailability not lower than of those which are already allowed (Brasil 2002). Ferric pyrophosphate and microencapsulated ferrous sulphate are

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also being employed to fortify wheat flour (Kiskini et al. 2010; Nabeshima et al. 2005).

After the fortification process, some stability parameters can be evaluated for ensure the quality of the raw material or final product, such as color, rheology, absorption capacity in the body, in addition to the pro-oxidant effect of metals, such as iron, on lipid oxidation (Akhtar et al. 2011; WHO 2006).

Color may be changed by the amount of bran reincorporated into the whole wheat flour, due to the presence of foreign material, addition of iron and the amount of pigment from wheat. Moreover, it is considered an important factor for the acceptance or rejection of the product for consumption (Akhtar et al. 2011; Rutz 2012).

Composition changes in wheat flour may occur naturally during storage (Rehman 2006). Acidity can be used as an indicator of quality of wheat flour during storage (Rutz 2012). The lipids in wheat flour, even in small percentages, can be altered by the hydrolysis and oxidation processes (Ortolan et al. 2008; Rutz 2012). The oxidation process consists of three stages: initiation, propagation, and termination. The initial reactions of lipid oxidation may occur through energy absorption and redox reaction. Redox reactions are catalyzed by transition metals, e.g., iron (Fenton/Haber–Weiss reactions), and are capable of breaking the electrochemical barrier between oxygen and unsaturated fatty acid molecules in the food, starting the oxidative process (Ferrari 1998). Since whole wheat flour has higher quantities of lipids and is fortified with different iron compounds, monitoring quality and/or stability during storage becomes necessary in order to verify possible changes that may occur.

The peroxide value (PV) is a parameter used to assess the quality and/or stability of wheat flour, because it is originated from the primary products of lipid oxidation, and may decrease the shelf life and nutritional value of foods (Silva and Silva 1999). The volatile compounds, ketones, alcohols, acids and aldehydes, such as hexanal, are products of the decomposition of the primary products of the oxidative process (peroxides) and many of these degradation compounds have an unpleasant odor (Silva and Silva 1999).

The evaluation of the hexanal, from lipid oxidation, is also used to assess the quality and/or stability of wheat flour. Currently, the solid-phase microextraction technique (SPME) has been employed for the extraction and analysis of volatile compounds, such as hexanal, in cereal flours (Cramer et al. 2005; Giannetti et al. 2014; Kaseleht et al. 2011) and the results have been expressed as area under the curve of the volatile compounds being analyzed (Bauer et al. 2013; Cramer et al. 2005).

Studies about different iron compounds and its effects in parameters of quality and/or stability of whole wheat flour

fortified with different iron compounds during storage are very important for evaluation of impacts of whole wheat flour fortification. Therefore, the objective of this study was to evaluate the effect of whole wheat flour fortification with different iron compounds on quality and/or stability parameters such as color, acidity, peroxide and hexanal value during the 120 days of storage. To verify the influence of each parameter (variable) evaluated on the quality of whole wheat flour fortified with different iron compounds, a principal components analysis (PCA) was performed.

Materials and methods

Preparation of samples and experimental design

For obtaining whole wheat flour (100 kg), 6% of wheat bran was incorporated into refined wheat flour (94%), (Moinho Guaçu Mirim, Mogi Guaçu, Brazil), and its composition was: 13.22 ± 0.11 ; 12.95 ± 0.42 ; 1.29 ± 0.01 ; 0.80 ± 0.02 ; 6.91 ± 0.31 and 84.95% of moisture, proteins, lipids, ashes, fibers and carbohydrates, respectively. Then, portions of 10 kg were fortified with 4.2 mg/100 g ($\pm 20\%$) in accordance with Normative Resolution 344 (Brasil 2002), with different iron compounds, totaling 6 samples and one control (without addition of iron). Iron compounds used were: monohydrated ferrous sulfate (FS) (M Cassab, SP, Brazil), ferrous fumarate (FF) (M Cassab, SP, Brazil), reduced iron (RI), (M Cassab, SP, Brazil); ferric sodium ethylenediaminetetraacetate (NaFeEDTA—Ferrazone), (Vogler Ingredients, SP, Brazil); microencapsulated ferrous sulfate (FSm), and microencapsulated ferrous fumarate (FFm), (Funcional Mikron, Valinhos, Brazil). In the process of fortification, each sample was homogenized in a twin shell blender (Tecnal, Piracicaba, Brazil), for 30 min, in portions of 5.0 kg. Portions of 1.0 kg were put in a plastic packages and stored at room temperature, for simulation of shelf life.

After the addition of different iron compounds in whole wheat flour, the parameters titratable acidity, color, peroxide value and hexanal were evaluated in the fortified and control samples, at the start time (T1) soon after the obtaining of the fortified flours. The iron content was evaluated before and after the process of fortification in the samples of whole wheat flour. After this period, the samples were kept in sealed packages, at room temperature, and every month a pack of each sample was opened and examined regarding titratable acidity, color, peroxide value and hexanal parameters for the monitoring of shelf life, for 120 days, and times were referred to as T2 (30 days), T3 (60 days), T4 (90 days) and T5 (120 days).

Physical–chemical analyses

All analyses were performed in three repetitions in each storage point.

Determination of iron content: The total iron content was determined in accordance with Rebellato et al. (2015).

Color analysis: Color determination was performed by parameters of luminosity L^* (ranging between 0 = black and 100 = white) and chromaticity coordinates a^* ($-a^*$ = green and $+a^*$ = red), and b^* ($-b^*$ = blue and $+b^*$ = yellow) using a MiniScan spectrophotometer (Hunterlab, Reston, United States), followed by the CIE-Lab system (Minolta 1993).

Lipid hydrolysis and oxidation parameters: Titratable acidity was measured according to the method described by Pizzinatto (1997) and peroxide value (PV) was determined in accordance to Jensen et al. (2011). For hexanal analysis 5 g of whole flour were taken and conditioned in amber glass bottles of 20 mL sealed with silicone covers coated with polytetrafluoroethylene (PTFE). The volatile compounds were extracted from the headspace of the samples using the technique of solid-phase microextraction (HS-SPME). The SPME fiber with Carboxen/polydimethylsiloxane coating (Car/PDMS) (75 $\mu\text{m} \times 10$ mm, Supelco, Bellefonte, PA, USA) was used for the extraction. The fiber was pre-conditioned according to the protocol suggested by the manufacturer. The bottles containing the samples were subjected to extraction for 40 min, in a water bath at 40 °C. Before extraction, each bottle underwent a pre-equilibration period at the same temperature of extraction, without fiber exposition for 10 min. After extraction, the fiber was removed and inserted into the chromatograph injector for thermal desorption of the analytes. **Chromatographic analysis:** After extraction, the volatile compounds were quantified with a Varian Star 3400CX gas chromatograph (CA, USA) equipped with a flame ionization detector (GC-FID). The fiber was thermally desorbed in the injection port at 250 °C for 10 min, in splitless mode during 2 min. The compounds were separated in a ZB-WAX Plus fused silica polar capillary column (60 m $\times$ 0.25 mm $\times$ 0.25 μm). Hydrogen was used as carrier gas at constant pressure (30 psi). The initial temperature of the column was adjusted to 35 °C and maintained during 10 min. Then a temperature gradient was initiated increasing at 3 °C/min up to 50 °C, followed by an increase of 4 °C/min up to 150 °C, increasing 10° C/min up to 210 °C and kept under isothermal conditions during 5 min. The detector temperature was maintained at 230 °C. A series of homologous n-alkanes were analyzed under the same chromatographic conditions to calculate the linear retention index (LRI). The qualitative analysis of the hexanal were conducted with a Shimadzu QP2010 Plus gas chromatograph attached to a

mass spectrometer (GC/MS, Shimadzu Corp., Kyoto, Japan). For these analyses, the same chromatographic conditions described above were used, and helium was used as carrier gas. The detector was operated in electron ionization mode with ionization energy of 70 eV and mass range of 35–350 m/z. The hexanal were identified based on the comparison with the mass spectra available in the National Institute of Standards and Technology (NIST) and on the comparison of the calculated LRI with those available in the scientific literature.

Data analysis

All data were analyzed using variance analysis (ANOVA) and the Tukey test ($p < 0.05$) for mean comparison using the Statistica 7.0 software (StatSoft, USA).

The exploratory data analysis was carried out by a Principal Components Analysis (PCA), using the Pirouette 3.11 program (Infometrix, Inc., Bothell, WA). In order to do so, the data were auto-scaled since the results obtained differed in order of magnitude.

Results and discussion

The iron content in the control sample was 1.38 mg/100 g. After the fortification process values were 5.39 ± 0.08 ; 5.64 ± 0.11 ; 4.80 ± 0.12 ; 5.74 ± 0.25 ; 6.29 ± 0.22 and 5.23 ± 0.09 mg/100 g, for the samples fortified with FS, FF, RI, NaFeEDTA, FS_m and FF_m, respectively. The values obtained from iron after fortification are in accordance with Normative Resolution 344 and coincide with the values reported by Rehman et al. (2006).

The results obtained for titratable acidity in control whole wheat flour and flour fortified with different iron compounds during storage are shown in Table 1.

At the initial time (T1), the acidity values ranged from 1.15 to 1.25 mL of NaOH/100 g and the control sample was significantly different ($p < 0.05$) from the sample fortified with NaFeEDTA only. After 30 days of storage (T2) the acidity values ranged from 1.61 to 2.21 mL/100 g and the control sample was not significantly different from the samples fortified with FF and RI. At T3, the values ranged from 1.35 to 1.84 mL/100 g and the control sample was significantly different from the samples fortified with NaFeEDTA and FF_m. At the 90th day of storage (T4), the acidity values ranged from 1.21 to 1.57 mL/100 g and the control sample was significantly different from the samples fortified with FS, RI and NaFeEDTA. At T5, the values ranged from 1.34 to 1.63 mL/100 g and the control sample was significantly different from whole wheat flour fortified with FS only. It is thus concluded that the acidity parameter was slightly affected when the different iron compounds,

Table 1 Titratable acidity values in whole wheat flour fortified with different iron compounds during 120 days of storage

WWF	Soluble alcohol acidity (mL/100 g sample)				
	T1	T2	T3	T4	T5
Control	1.15 ± 0.03b,D	2.13 ± 0.01b,A	1.46 ± 0.02b,c,B	1.23 ± 0.03c,C	1.42 ± 0.01b,c,B
FS	1.18 ± 0.03b,E	1.79 ± 0.02c,A	1.48 ± 0.02b,c,C	1.35 ± 0.04b,D	1.63 ± 0.06a,B
FF	1.15 ± 0.01b,E	2.17 ± 0.02a,b,A	1.50 ± 0.04b,B	1.26 ± 0.02b,c,D	1.40 ± 0.03b,c,C
RI	1.17 ± 0.02b,D	2.13 ± 0.02b,A	1.43 ± 0.04b,c,d,B,C	1.53 ± 0.07a,B	1.38 ± 0.04b,c,C
NaFeEDTA	1.25 ± 0.01a,D	2.21 ± 0.04a,A	1.84 ± 0.02a,B	1.57 ± 0.02a,C	1.47 ± 0.07b,C
F _{Sm}	1.17 ± 0.00b,E	1.61 ± 0.01d,A	1.41 ± 0.04c,d,B	1.24 ± 0.02c,D	1.34 ± 0.01c,C
F _{Fm}	1.19 ± 0.02a,b,C	1.63 ± 0.03d,A	1.35 ± 0.02d,B	1.21 ± 0.03c,C	1.41 ± 0.04b,c,B

Mean ± standard deviation. WWF whole wheat flour, Control: whole wheat flour without addition of iron, FS ferrous sulphate, FF ferrous fumarate, RI reduced iron, NaFeEDTA ferric sodium ethylenediaminetetraacetate, F_{Sm} microencapsulated ferrous sulfate, F_{Fm} microencapsulated ferrous fumarate. T1: start; T2: 30 days; T3: 60 days; T4: 90 days; T5: 120 days. Means with different lowercase letters in the same column indicate significant difference between samples ($p < 0.05$). Means with different uppercase letters in the same row indicate significant difference between times ($p < 0.05$)

principally NaFeEDTA, were employed in the process of fortification, which changed negatively the quality of the flour, when compared with the control.

Regarding the influence of titratable acidity over time for each sample, it is possible to verify that the samples had the same behavior; at T1 samples showed a lower value of acidity, T2 was the period in which the samples showed the highest value of acidity, at T3 and T4 a reduction in the evaluated values occurred and at T5, a slight increase in the value of acidity was verified for most samples.

Rehman (2006) assessed the effects of storage in the nutritional quality of cereals. The authors found that the acidity values in wheat ranged from 2.85 to 4.00 mg NaOH/100 g, during the 6-month storage. Miranda and El-Dash (2002) monitored the stability of germinated whole wheat flour during 6 months of storage, and found that the acidity values increased during the evaluated period, for both control and germinated samples, and attributed this occurrence to the gradual hydrolysis of triacylglycerol with formation of free fatty acids during storage. The increased acidity, caused by the hydrolysis of fats and the formation of free fatty acids during storage, is related to the initial stage of the loss of quality of the flours (Araújo 2004; Pomeranz 1974).

Regarding color parameters of whole wheat flour, the values of L^* , a^* and b^* were evaluated both for control and samples fortified with different iron compounds (Table 2).

The values of L^* , a^* and b^* in the samples at T1, T2, T3, T4 and T5 were evaluated and significant difference was not verified ($p > 0.05$) between control sample and samples fortified with different iron compounds, i.e. regardless of the form of iron used in the fortification of whole wheat flour, changes in the color parameters were not verified. The wheat bran used in the preparation of

whole wheat flour presented darker color and the presence of iron, became imperceptible (Alam et al. 2009).

The evaluation of color during storage time for each sample, it is possible to verify that the flours showed the same behavior for parameter L^* . At T1 and T2 the values did not change, with the exception of the sample fortified with micro FF which showed a decrease in parameter L^* at T2. At T3, all samples presented higher value of L^* , a slight decrease was verified at T4 and T5 had no alterations. Regarding coordinate a^* , significant difference was not verified ($p > 0.05$) over time for any of the samples evaluated. For coordinate b^* , the control samples, FS and RI presented the same behavior over time, i.e. the yellow coloring remained stable until T3 and decreased at T4, with no significant difference ($p > 0.05$) at T5. The flour fortified with FF had a color change only at T5. Flours fortified with F_{Sm} and F_{Fm} had a decrease in the yellow coloring at T3 and T2, respectively; changes at other times were not verified.

Parameters L^* and b^* were influenced by time of storage, which may be associated with the reduction of free carotenoids, specially luteins, which are naturally present in wheat flour. Gutkoski et al. (2008) evaluated the effect of fortification of wheat flour with reduced iron for a period of 180 days and obtained results similar to our study, in which there was an increase in parameter L^* and a decrease in b^* over the period assessed. Alam et al. (2009) also verified changes in the coloring of whole wheat flour fortified with Fe^{2+} .

The peroxide values were assessed during the 120 days of storage for control and samples fortified with different iron compounds and are presented in Table 3.

At T1 the control sample was not significantly different ($p > 0.05$) from samples fortified with FF, NaFeEDTA and

Table 2 Values of color parameters: luminosity L^* and coordinates a^* and b^* in whole wheat flour fortified with different iron compounds during 120 days of storage

	T1	T2	T3	T4	T5
WWF	<i>PARAMETER L^*</i>				
Control	93.06 ± 0.14C	93.10 ± 0.03C	94.45 ± 0.05A	93.86 ± 0.07B	93.99 ± 0.27B
FS	93.14 ± 0.04C	92.90 ± 0.15C	94.36 ± 0.22A	93.68 ± 0.22B	94.03 ± 0.10A,B
FF	93.05 ± 0.21C,D	92.99 ± 0.15D	94.39 ± 0.19A	93.61 ± 0.32B,C	94.04 ± 0.21A,B
RI	93.33 ± 0.32C,D	93.04 ± 0.22D	94.72 ± 0.09A	93.82 ± 0.12B,C	93.99 ± 0.20B
NaFeEDTA	93.11 ± 0.18C	93.16 ± 0.20C	94.54 ± 0.14A	93.76 ± 0.04B	94.02 ± 0.14B
FSm	93.15 ± 0.40D	93.31 ± 0.15C,D	94.48 ± 0.09A	93.69 ± 0.09B,C	93.92 ± 0.07B
FFm	93.56 ± 0.03B	93.17 ± 0.13C	94.39 ± 0.25A	93.81 ± 0.07B	93.90 ± 0.14B
Whole WF	<i>COORDINATE a^*</i>				
Control	0.92 ± 0.07A	0.84 ± 0.06A	0.87 ± 0.03A	0.82 ± 0.03A	0.89 ± 0.04A
FS	0.94 ± 0.07A	0.92 ± 0.06A	0.92 ± 0.04A	0.82 ± 0.05A	0.82 ± 0.04A
FF	0.96 ± 0.06A	0.93 ± 0.06A	0.93 ± 0.08A	0.89 ± 0.06A	0.84 ± 0.06A
RI	0.83 ± 0.10A	0.89 ± 0.06A	0.84 ± 0.05A	0.85 ± 0.07A	0.88 ± 0.02A
NaFeEDTA	0.93 ± 0.10A	0.91 ± 0.03A	0.89 ± 0.05A	0.88 ± 0.04A	0.84 ± 0.03A
FSm	0.93 ± 0.05A	0.84 ± 0.03A	0.92 ± 0.07A	0.90 ± 0.07A	0.86 ± 0.03A
FFm	0.88 ± 0.03A	0.85 ± 0.04A	0.88 ± 0.04A	0.87 ± 0.05A	0.84 ± 0.02A
Whole WF	<i>COORDINATE b^*</i>				
Control	10.25 ± 0.24A	10.06 ± 0.10A,B	9.95 ± 0.07A,B	9.74 ± 0.20B	9.70 ± 0.22B
FS	10.28 ± 0.24A	9.97 ± 0.22A,B	9.75 ± 0.07A,B,C	9.43 ± 0.24B,C	9.18 ± 0.35C
FF	10.27 ± 0.21A	10.03 ± 0.11A,B	9.87 ± 0.36A,B	9.73 ± 0.14A,B	9.59 ± 0.29B
RI	10.29 ± 0.12A	10.19 ± 0.12A,B	9.94 ± 0.24A,B,C	9.83 ± 0.06B,C	9.67 ± 0.11C
NaFeEDTA	10.19 ± 0.05A	9.75 ± 0.40A	9.99 ± 0.20A	9.84 ± 0.12A	9.54 ± 0.40A
FSm	10.33 ± 0.14A	10.16 ± 0.18A,B	9.88 ± 0.12B,C	9.54 ± 0.10C,D	9.52 ± 0.06D
FFm	10.29 ± 0.11A	9.89 ± 0.08B	9.82 ± 0.06B,C	9.63 ± 0.23B,C	9.48 ± 0.09C

Mean ± standard deviation. *Whole WF* whole wheat flour, Control: whole wheat flour without addition of iron, *FS* ferrous sulphate, *FF* ferrous fumarate, *RI* reduced iron, *NaFeEDTA* ferric sodium ethylenediaminetetraacetate, *FSm* microencapsulated ferrous sulfate, *FFm* microencapsulated ferrous fumarate. T1: start; T2: 30 days; T3: 60 days; T4: 90 days; T5: 120 days. Means with different uppercase letters in the same row indicate significant difference between times ($p < 0.05$)

Table 3 Peroxide value in whole wheat flour fortified with different iron compounds during 120 days of storage

WWF	Peroxide value (microequivalent of hydrogen peroxide/kg of sample)				
	T1	T2	T3	T4	T5
Control	9.90 ± 0.50b,C	14.80 ± 0.70a,b,c,A	14.00 ± 0.80b,A	11.10 ± 0.40b,c,B	11.40 ± 0.60c,B
FS	7.20 ± 0.80c,C	15.7 ± 0.50a,b,A	15.40 ± 0.70a,A	11.90 ± 0.50a,b,B	12.80 ± 0.70a,b,B
FF	8.8 ± 1.00b,c,C	13.7 ± 0.60b,c,A	14.10 ± 1.00b,A	12.40 ± 0.60a,B	13.40 ± 0.20a,A,B
RI	12.00 ± 1.50a,B,C	13.00 ± 1.10c,A,B	13.70 ± 0.60b,A	11.00 ± 0.60b,c,C	12.50 ± 0.60a,b,A,B,C
NaFeEDTA	9.60 ± 0.70b,C	9.10 ± 1.30d,C	14.30 ± 0.80a,b,A	12.40 ± 0.90a,B	12.50 ± 0.80b,c,B
FSm	4.30 ± 0.40d,D	6.40 ± 0.50e,C	14.20 ± 0.40a,b,A	11.00 ± 0.70b,c,B	11.80 ± 0.50b,c,B
FFm	9.6 ± 1.30b,C	16.50 ± 2.20a,A	14.00 ± 0.50b,B	10.00 ± 0.70c,C	12.50 ± 0.70a,b,B

Mean ± standard deviation. *WWF* whole wheat flour, Control: whole wheat flour without addition of iron, *FS* ferrous sulphate, *FF* ferrous fumarate, *RI* reduced iron, *NaFeEDTA* ferric sodium ethylenediaminetetraacetate, *FSm* microencapsulated ferrous sulfate, *FFm* microencapsulated ferrous fumarate. T1: start; T2: 30 days; T3: 60 days; T4: 90 days; T5: 120 days. Means with different lowercase letters in the same column indicate significant difference between samples ($p < 0.05$). Means with different uppercase letters in the same row indicate significant difference between times ($p < 0.05$)

Table 4 Hexanal area in whole wheat flour fortified with different iron compounds during 120 days of storage

WWF	Hexanal Area*				
	T1	T2	T3	T4	T5
Control	4.86 ± 0.14c,D	15.62 ± 1.69d,e,C	47.23 ± 1.89d,A	46.79 ± 1.03e,A	39.48 ± 0.47c,B
FS	122.71 ± 5.08b,D	320.49 ± 4.27a,A	239.23 ± 8.40a,B	179.16 ± 6.55b,C	112.59 ± 5.61b,D
FF	6.08 ± 0.65c,D	23.38 ± 1.46d,C	59.85 ± 1.02c,A	61.15 ± 1.15d,A	43.66 ± 2.31c,B
RI	5.60 ± 0.38c,D	22.32 ± 0.45d,e,C	42.17 ± 1.39d,e,B	50.46 ± 1.82e,A	44.28 ± 2.20c,B
NaFeEDTA	6.50 ± 1.51c,E	14.58 ± 0.91e,D	31.25 ± 1.10e,C	52.55 ± 2.97d,e,A	47.79 ± 1.54c,B
F5m	130.56 ± 4.40a,D	150.40 ± 5.59b,C	176.37 ± 5.01b,B	211.66 ± 1.16a,A	181.00 ± 5.46a,B
FFm	7.40 ± 0.78c,E	31.31 ± 0.58c,D	67.01 ± 3.16c,C	97.08 ± 4.54c,B	116.56 ± 1.74b,A

Mean ± standard deviation. *Hexanal area $\times 10^4$. WWF whole wheat flour, Control: whole wheat flour without addition of iron, FS ferrous sulphate, FF ferrous fumarate, RI reduced iron, NaFeEDTA ferric sodium ethylenediaminetetraacetate, F5m microencapsulated ferrous sulfate, FFm microencapsulated ferrous fumarate. T1: start; T2: 30 days; T3: 60 days; T4: 90 days; T5: 120 days. Means with different lowercase letters in the same column indicate significant difference between samples ($p < 0.05$). Means with different uppercase letters in the same row indicate significant difference between times ($p < 0.05$)

FFm, and the flour fortified with RI had the highest PV. After 30 days of storage, the control sample only differed from the samples fortified with NaFeEDTA and F5m. At T3, only the flour fortified with FS was significantly different ($p < 0.05$) from the control. At T4, the PV of the control flour was significantly different from the samples fortified with FF and NaFeEDTA, and at T5, only the samples fortified with NaFeEDTA and F5m were not significantly different from the control sample.

Regarding evaluation over time for each sample, the PV for the control samples and those fortified with FS and FF showed the same behavior, that is, the higher peroxide values were verified at T2, remained stable at T3, declined at T4 and continued stable at T5. The samples fortified with RI, NaFeEDTA and F5m present higher PV at T3, while the whole wheat flour fortified with FFm had the highest PV at T2.

Rehman et al. (2006) evaluated the stability of Fe^{2+} in fortified whole wheat flour (FeSO_4 , EDTA and folic acid) during 42 days of storage. The authors reported that the samples did not show significant difference in relation to the total iron content, however, a conversion of Fe^{2+} into Fe^{3+} was observed and indicated that oxidation occurs during the period of storage. Alam et al. (2009) studied the stability of whole wheat flour containing FeSO_4 , EDTA and folic acid during 60 days of storage and verified reduction of Fe^{2+} and an increase in Fe^{3+} during the evaluated period due to the oxidation of iron.

Chung (2009) studied the influence of addition of purple sweet potato flour on the oxidative stability of cookies during 80 days at 60 °C and noted that both the values of acidity and peroxide increased during the storage period. Jensen et al. (2011) assessed the stability of whole wheat bread for 5 weeks of storage and used several markers of

lipid oxidation, including PV. The authors found that the hydroperoxide content originated from the primary oxidation of lipids was higher in the crumb of the bread than in the crust, and that storage also had a significant effect on PV, mainly between the second and third week of storage. Altunkaya et al. (2013) investigated the oxidative stability of bread enriched with different percentages of pomegranate peel flour, and found that the concentration of lipid peroxides (PV) increased significantly in all samples of bread during storage.

The results obtained for hexanal area in control sample and samples fortified with different iron compounds, during storage, are presented in Table 4.

At the initial time (T1), only the control sample was significantly different ($p < 0.05$) from the samples fortified with FS and F5m, and these samples were the ones with the highest values of hexanal area. After 30 days of storage, the control sample was only different from the samples fortified with FS, F5m and FFm, which were also the samples with the highest values of hexanal area. At T3, the samples fortified with FS, FF, NaFeEDTA, F5m and FFm were the flours with the highest values of hexanal area and differed significantly from the control sample. At the 90th day of storage (T4), the control sample did not differ from the samples fortified with RI and NaFeEDTA, and the samples fortified with FS and F5m presented higher values of hexanal area. At the 120th day of storage (T5), the control sample was only significantly different from the samples fortified with FS, F5m and FFm, which were also the samples with the highest values of hexanal area.

When assessing the values of hexanal area in the samples during storage time, it is possible to verify that the control sample presented the highest value at T3 (60 days) and T4 (90 days). The same behavior was verified in the

whole wheat flour fortified with FF. The sample fortified with FS presented the highest hexanal value soon after 30 days of storage, while the sample fortified with FFm had the highest value at T5 (120 days). The other samples (RI, NaFeEDTA and SFm) had the highest hexanal values at the 90th day of storage (T4). Thus, it is verified that the different iron compounds used in fortification influenced negatively the stability of the samples of whole wheat flour.

Cramer et al. (2005) analyzed volatile compounds in barley and wheat using the SPME extraction technique and found hexanal only in the samples of barley. Giannetti et al. (2014) used the HS-SPME technique followed by GC/MS to assess the volatile fraction of mass made with flour of hard wheat produced under different conditions of processing and verified the presence of some lipid oxidation products, including hexanal.

There are few studies in the scientific publications about oxidative stability of whole wheat flour fortified with different iron compounds during storage, which makes it difficult to compare the results. However, Hurrell et al. (2010) published recommendations for the fortification of wheat flour with iron and argue that NaFeEDTA must be used as it does not promote oxidation in flour during storage. Electrolytic iron is not very soluble, but it does not promote sensory changes in foods. Ferrous fumarate should have less impact on the sensory evaluation when compared to ferrous sulphate, and the encapsulated compounds should prevent lipid oxidation of the flour during storage.

In this study it was found that NaFeEDTA promoted minor changes in whole flours stability, however changes in acidity parameters, PV and hexanal values were observed when compared with the control.

The whole wheat flours fortified with FS and FS_m presents not only high reactivity, but also high pro-oxidant potential, which may have favored the development of the oxidative process in the flour during the 120 days of storage, consequently obtaining a higher value in the hexanal area. Contrary to expectations, the microencapsulated compounds studied promoted changes in the parameters assessed. The microencapsulated compounds should have protected the whole wheat flour fortified from the developing the oxidative process during storage, due to the positive characteristics that the encapsulation process proposes. However this effect was not verified, more detailed studies should be conducted for further clarification on the effectiveness of microencapsulation.

In order to verify the correlations between the studied variables and the relationships with the different iron compounds used in the fortification of the samples of whole wheat flour, a principal components analysis (PCA) was performed. A data matrix (6 variables × 35 samples) was built using the following variables: iron content,

titratable acidity, color coordinates L* and b* (the color parameter a* was not used since it was not detected differences among samples), PV and hexanal values, as column, and samples as lines. Three main components were used to describe 76.28% of the total variance. The results obtained from the PCA for the parameters evaluated in whole wheat flour fortified with different iron compounds and in the control sample are shown in Fig. 1, being (a) the scores graph and (b) the loading plot.

The dominant variables for the first principal component (PC1) were: color parameters L* and PV (positively correlated), b* (negatively correlated), having described 32.65% of the total variance of the results. The second main component (PC2) was responsible for describing 24.30% of the total variance with the exception of the color

Fig. 1 Principal Component Analysis for quality parameters of control whole wheat flour and flour fortified with different iron compounds, **a** scores graph (samples), **b** loading graph (variables). Control: whole wheat flour without addition of iron; FS ferrous sulphate, FF ferrous fumarate, RI reduced iron, EDTA ferric sodium ethylenediaminetetraacetate (NaFeEDTA), FS_m microencapsulated ferrous sulfate, FF_m microencapsulated ferrous fumarate. 1: T1 (start); 2: T2 (30 days); 3: T3 (60 days); 4: T4 (90 days); 5: T5 (120 days)

variables (L^* and b^*), whereas all the others contributed to the variability of the results. PC3 described 19.32% of the variance and the acidity variables (positively correlated) and coordinate L^* (negatively correlated) were those that contributed most to the variability of the samples.

In the score plot (a) it is possible to verify that grouping occurred between the samples. The control samples are at the bottom of the scores graph and presented the lowest iron and hexanal values. The samples that were fortified with SF3, SF4, SF5, SFm3, SFm4, SFm5, FFm3, FFm5, in the respective times, were the samples with the highest values of iron and hexanal, i.e. the storage time for whole wheat flour fortified with these iron compounds is directly influenced by lipid oxidation, due to the higher values of hexanal. The whole wheat flours fortified with the different iron compounds were the samples with the least influence in relation to the parameters evaluated at T1 and T2.

Conclusion

The fortification of whole wheat flour with different iron compounds changed the quality parameters and/or stability for studied samples.

Flours fortified with FS, FSm and FFm were the ones with most changes in the parameters evaluated. For the whole flours fortified with NaFeEDTA and RI compounds, minor changes were found during storage time; therefore, these compounds were considered more stable and showed behavior similar to the control sample when used in the process of fortification during storage.

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