

**EFFECT OF LACTIC FERMENTATION ON NUTRITIONAL
POTENTIAL OF MULTIGRAIN FLOURS BASED ON WHEAT, RYE AND
OAT**

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Received on 10 January 2019

Revised on 18 February 2019

This study was focused on the multigrain flours obtained by milling blends with various amounts of wheat, rye and hulled oat, and on the sourdoughs prepared out of these flours using different commercial starter culture. The folate and total phenol levels and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA) of multigrain flours increased with the addition level of rye and hulled oat. Among the fractions obtained through milling, higher levels of folates were registered in brans, followed by shorts and then by flours. The folate contents in brans and shorts were about of 1-1.4 times higher than in the flours. The sourdough fermentation realized with different commercial starter cultures induced the increase of the total phenols content and DPPH-RSA. The highest levels of folates were obtained when fermenting the flour suspensions with *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*, followed by samples with *Lactococcus lactis* subsp. *Lactis* and *Leuconostoc*. The sourdough fermented with *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum* had lower folate contents compared to the corresponding controls obtained through spontaneous fermentation. The obtained results suggest that both the type of cereals used to obtain the flours and the starter culture used to prepare the sourdoughs are suitable tools which worth be used for modulating the nutritional profile of the baking products.

Keywords: multigrain milling fractions, total phenol content, antioxidant activity, folates, sourdough

Introduction

Cereals are important sources of phytochemicals, including folates and phenolic acids. The content of such phytochemicals varies with the type of cereal. Different studies available in the literature indicate that the folate content in cereals decrease in following order: barley (717.5ng/g) > rye (693 ng/g) > oat (565.8 ng/g) > wheat

(561 ng/g), while the mean of total phenolic content decrease in following order: rye (693 µg/g) > wheat (664 µg/g) > oat (531 µg/g) > barley (463.12 µg/g) (Andersson *et al.*, 2008; Nystrom *et al.*, 2008; Li *et al.*, 2008; Shewry *et al.*, 2008).

The reason for cereals milling is to separate the starchy endosperm by the aleurone, pericarp layers and germ, followed by endosperm grinding into flour. Taking into account that the most important nutritional components are located in the aleurone layer, through the milling process these components are mainly directed towards the bran. Anyway, depending on the extraction rate of the flours, different proportions of aleurone layers might participate to the formation of flours. Therefore, the extraction rate has a high influence on the nutritional value of flours. According to Dewettinck *et al.* (2008), the content of phytochemicals, minerals and total fiber rate are directly related to the flours extraction. Therefore, the nutritional components of flours can be modulated by choosing a certain extraction rate, in such way to get technological properties of flours suitable for the type of bakery products that are to be obtained. The improvement of the nutritional potential of flours can be achieved by blending different types of flours, usually the wheat flour with various percentages of flours from other cereals, pseudo-cereals or vegetables, to obtain the composite flours. A less studied alternative, recently presented by Tulse *et al.* (2014), consists in blending the cereals followed by their milling to obtain multigrain flours.

Regardless of the procedure used to obtain the flours, the use of sourdough fermentation stage during the breadmaking process is a good opportunity to improve the bakery products quality, including the phytochemicals bioavailability (Poutanen *et al.*, 2009).

The objective of this study was to investigate the effect of sourdough fermentation on the folate and phenolic acid contents and on antioxidant activity of multigrain flours. Different commercial starter cultures consisting of lactic acid bacteria (LAB) and multigrain flours based on wheat with different addition levels of rye and hulled oat were used to prepare the sourdoughs.

Materials and Methods

Materials

Wheat (Boema variety; 13.4% protein, 2.1% fat, 1.65% ash), rye (Suceveana variety; 9.8% protein, 1.8% fat, 1.75% ash) grown in South East Romanian Plain, harvested in 2016, and hulled oat (13.8% protein, 5.2% fat, 1.92% ash) purchased from Galati (Romania) market were used in the experiments.

Three different commercial strains were used for preparing the sourdoughs out of the multigrain flours: starter culture 1 (SC1) - DI-PROX MTTX1 from EDR Ingredients (Romania) consisting of a mixture of *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum*; starter culture 2 (SC2) - YF-811 from Chr. Hansen (Romania) consisting of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*; starter culture 3 (SC3) - CHN-22 from Chr. Hansen (Romania) consisting of *Lactococcus lactis* subsp. *Lactis* and

Leuconostoc. The inoculum size used for preparing the sourdoughs was according to producer recommendations.

Multigrain flours preparation

Wheat (W), rye (R) and hulled oat (O) were blended in the following ratios: 80:10:10 (80W+10R+10O), 70:15:15 (70W+15R+15O) and 60:20:20 (60W+20R+20O), followed the milling using the Buhler laboratory mill MLU (Buhler, Uzwil, Switzerland), according to the procedure described by Aprodu and Banu (2017), and Aprodu *et al.* (2017). For each cereal mixture three milling products were collected as follows: one multigrain flour (F10, F15 and F20 obtained from cereal blends including 10, 15 and 20% rye and hulled oat, respectively), one bran stream (B10, B15 and B20 obtained from cereal blends including 10, 15 and 20% rye and hulled oat, respectively) and one short stream (S10, S15 and S20 obtained from cereal blends including 10, 15 and 20% rye and hulled oat, respectively). The multigrain flours formed through milling the three multigrain blends, 80W+10R+10O (F10), 70W+15R+15O (F15) and 60W+20R+20O (F20), had extraction rates decreasing with the increase of the wheat substitution level as follows: 72.2%, 69% and 66.2%, respectively.

Sourdough preparation

In order to obtain the sourdoughs, the multigrain flours were first mixed with tap water (ratio 1:2) to obtain a dough yield of 300. After inoculating the multigrain flour suspensions with starter culture, fermentation was carried out for 20 hours at 30°C. For all tested multigrain flours, control samples (C10, C15 and C20 obtained with multigrain flours having 10, 15 and 20% of rye and hulled oat) were prepared without starter cultures addition, by spontaneous fermentation under the condition specified for the sourdough samples. Samples codification is provided in Table 1.

Table 1. Codification of the controls and sourdough samples used in the experiment

Multigrain flour	Starter culture			
	Without	SC1	SC2	SC3
F10	C10	SC1_10	SC2_10	SC3_10
F15	C15	SC1_15	SC2_15	SC3_15
F20	C20	SC1_20	SC2_20	SC3_20

Multigrain flours and sourdough characterization

The total phenolic content (TPC) was quantified using the Folin Ciocalteu method described by Singleton and Rossi (1965) and modified by Gao *et al.* (2002) and was expressed as mg ferulic acid equivalent (FAE) per g d.w.

The antioxidant activity was quantified as the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH RSA), using the method described by Brand-Williams *et al.* (1995) and modified by Beta *et al.* (2005).

The pH of the sourdoughs was measurement according to SR ISO 90/2007 (ASRO 2008), using a Hanna digital pH-meter.

The content of lactic and acetic acids of sourdough samples were determined using dedicated R-Biopharm kits (Boehringer Mannheim). Producer recommendation was strictly followed for lactic and acetic acids quantification.

The content of folic acid was determined using the competitive enzyme immunoassay Ridascreen®Fast Folic Acid (R-Biopharm Rhone Ltd.) specially designed for the quantification of folic acid in foods. The procedure indicated by the manufacturer was followed. The optical density of the microtiter plate was measured using a plate reader (Stat Fax® 4700, Awareness Technology, Inc.) and the software RIDA® Soft Win (R-Biopharm AG, Germany) was used for folic acid content quantification.

Statistical analysis

The experiments were carried out in triplicate. Significant differences among results were determined through analysis of variance, performed with Microsoft Excel software.

Results and discussion

Characterization of the multigrain milling fractions

The fractions obtained through milling the multigrain mixtures with different percentages of wheat, rye and hulled oat were characterized in terms of antioxidant activity, TPC and folate content. In Figure 1 are presented the TPC and DPPH-RSA results on multigrain flours, brans and shorts.

The levels of TPC varied from 240.57 to 321.17 mg FAE/g d.w. in flours with increasing contents of rye and hulled oat in the blends. The highest TPC values were found in shorts arising from the cereal blends with 10 and 15% rye and hulled oat (Figure 1a). In case of the blends with 20% rye and hulled oat, the highest amount of phenolic compounds was directed toward bran, suggesting that during milling a part of the aleurone layer, especial from hulled oat kernels, remained attached to the outer layers. This observation is in agreement with Aprodu and Banu (2017) who noted that the bran stream resulting from oat milling has flattened and large particles with large amounts of adherent endosperm.

The trend registered for DPPH-RSA was similar to the TPC; higher DPPH-RSA values were found for brans and shorts compared to the flours. Anyway, unlike the TPC values, the antioxidant activity of the flours is closer of those of the corresponding brans (Figure 1). In addition, one can note that, for all studied multigrain blends subjected to milling, the DPPH-RSA values were higher for shorts than for corresponding bran fractions. These results might be due to the presence of the hulled oat within blends. According to Hitayezu *et al.* (2015) the polyphenolic compounds from oat are phenolic acids, flavonoids and avenanthramides, and Emmons *et al.* (1999) reported that the main avenanthramides present a strong scavenging activity. Hitayezu *et al.* (2015) found a significant correlation between avenanthramides and radical scavenging activity, but not between avenanthramides and phenolic acids, suggesting the great importance of the contribution of avenanthramides to the antioxidant activity.

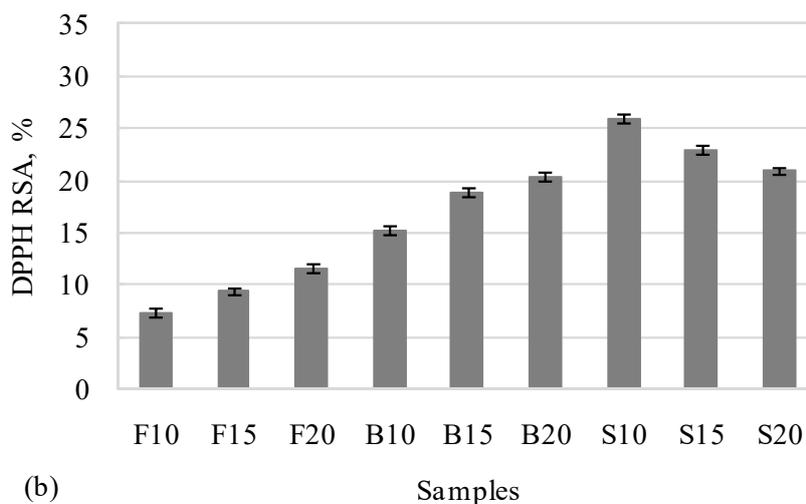
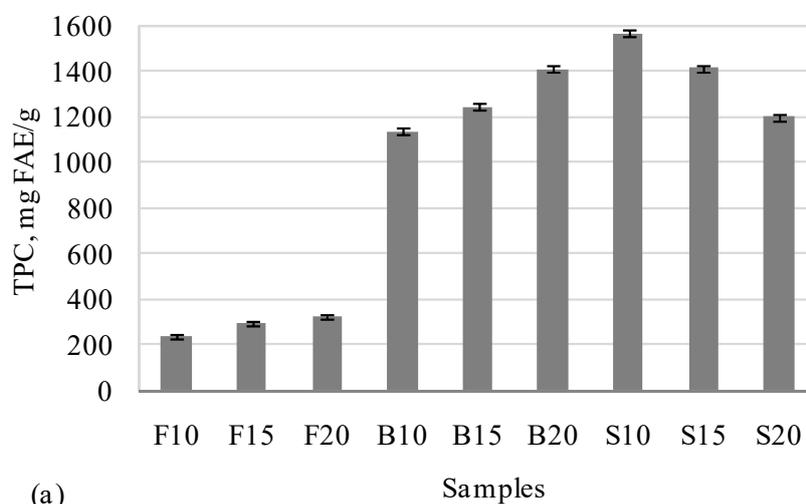


Figure 1. Total phenol content (a) and DPPH-RSA (b) of multigrain milling fractions

Regarding the folate contents in the multigrain milling fractions, our results indicated the increase of the folates amount present in the flours and shorts with increasing the wheat substitution level by rye and hulled oat within blends (Figure 2). These observations are in agreement with Nystrom *et al.* (2008), Li *et al.* (2008) and Shewry *et al.* (2008), who reported that rye has higher folates content than oats and even higher than wheat.

Our results indicated the highest folate levels in shorts, followed by brans and then by flours. In case of wheat, Arcot *et al.* (2002) reported that the highest content of

folates is located in the aleurone layer, and there is a significant correlation between ash and folate contents. On the other hand, when studying the oat grains, Edelmann *et al.* (2012) showed that the content of folates in the endosperm is about 0.7-0.8 times lower compared to the whole oat, while in bran there is about 1.3-1.5 times higher than in the whole oat. Moreover, they reported no significant correlation between ash and folates contents in oat milling fractions. Our results indicated that in case of milling multigrain blends including wheat, rye and hulled oat, the content of folates in the bran and short fractions is about 1.3-1.4 and 1-1.3 times higher, respectively compared to the corresponding flours.

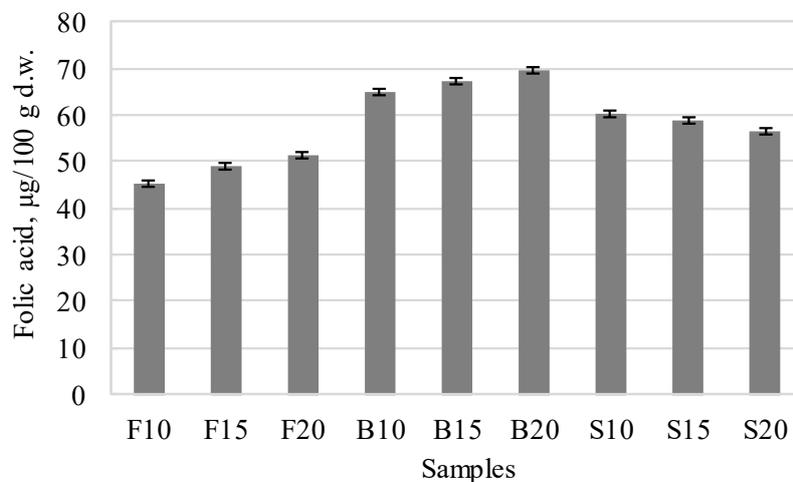


Figure 2. Folates contents of multigrain milling fractions

Characterization of the sourdough

The multigrain flours were used to obtain sourdoughs through fermentation for 20h at 30°C. At the end of this period, the intensity of lactic acid bacteria metabolic activity was estimated by quantifying the main fermentation metabolites.

The lactic acid and acetic acid contents of sourdoughs increased with the wheat substitution level by rye and hulled oat in the blends, both in control samples (C) and samples fermented with culture starter (SC1, SC2 and SC3) (Figure 3a).

The highest amounts of lactic acid were found in the sourdoughs prepared with SC3 followed by the ones prepared with SC2, while the lower values were registered for control samples. Regarding the acetic acid, higher contents were obtained in case of control samples, followed the sourdough prepared with SC1, while the lowest value was obtained for sourdough prepared with SC2 (Figure 3a). Moreover, the sourdough prepared with SC2 had the lowest pH and the highest lactic acid/acetic acid ratio (Figure 3b). In case of all controls and sourdough samples prepared with SC1 and SC3, the lactic acid/acetic acid ratio increased with the levels of rye and hulled oat within cereal blends, while in case of sourdough

prepared with SC2 the lactic acid/acetic acid ratio decreased with increasing the wheat substitution level.

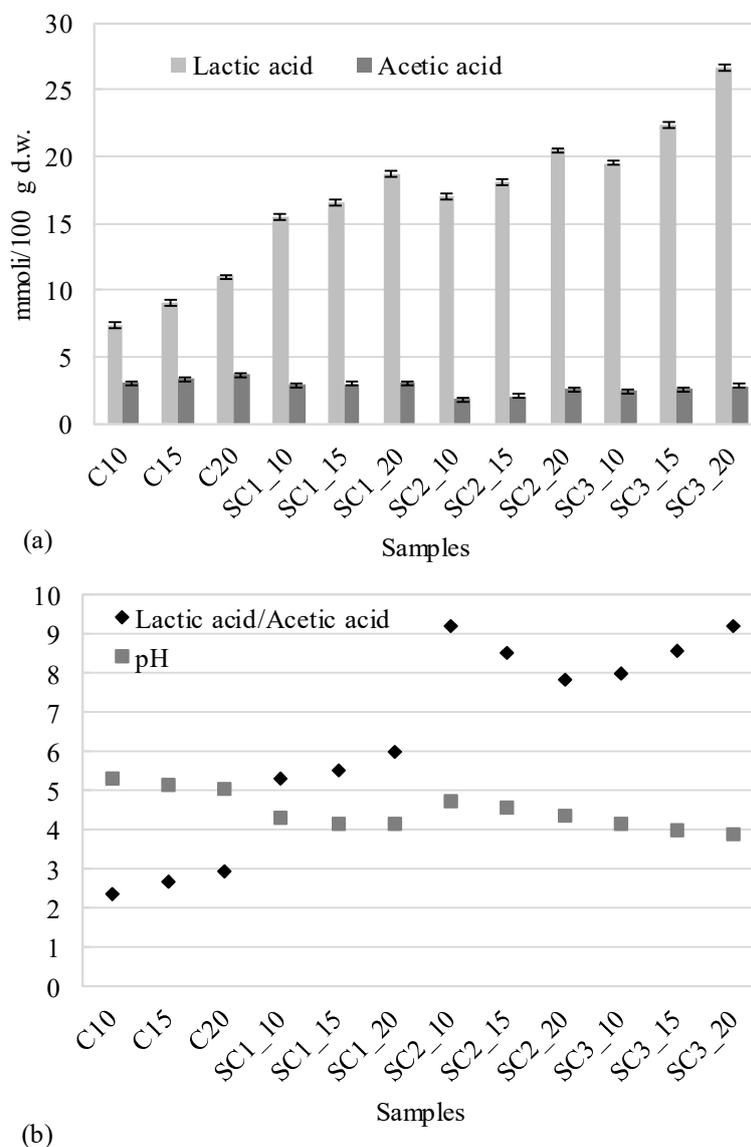


Figure 3. The production of lactic acid and acetic acid (a), lactic acid/acetic acid and pH (b) of during sourdoughs prepared without starter culture (C) and sourdoughs fermented with starter culture (SC)

The higher lactic acid/acetic acid ratio obtained in case of sourdough samples prepared with SC2 and SC3 might be explained by the fact that these starter cultures include homofermentative LAB, such as *Streptococcus thermophiles* and *Lactococcus lactis*, respectively. When compared to the corresponding multigrain

flours, the sourdough fermentation resulted in the increase of TPC and DPPH-RSA (Figure 1 and Figure 4). According to Dordevic *et al.* (2010) and Banu *et al.* (2010, 2011) the fermentation create condition for the increase of enzymatic activity of the endogenous enzyme, that act on cereal cell walls, causing the release and/or synthesis of some bioactive compounds.

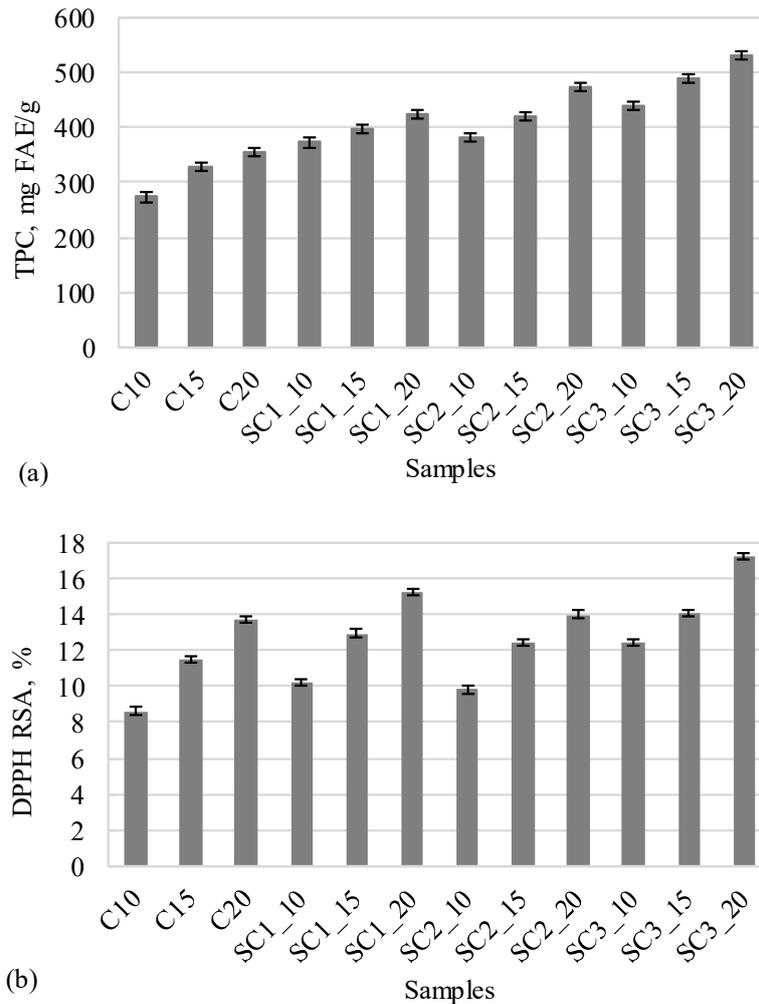


Figure 4. The total phenolic contents (a) and antioxidant activity (b) of the sourdoughs prepared without starter culture (C) and sourdoughs fermented with starter culture (SC)

Taking into account that the optimum pH values of enzymes acting on cell walls are different, Dordevic *et al.* (2010) consider that the type of fermentation and the LAB species influence the accumulation of bioactive compounds in sourdough. Our results indicated higher TPC and DPPH-RSA values for sourdough fermented with SC1 and SC3. These sourdough samples had the lower pH values after

fermentation (Figure 3), most probably causing the increase of the levels of easily extractible phenolic compounds (Banu *et al.*, 2010).

The ability of LAB to produce folates varies from one strain to another. Different strains of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Streptococcus thermophilus* and *Lactococcus lactis* have the ability to produce folates, but some of them might equally consume the folate (Capozzi *et al.*, 2012). According to Kariluoto *et al.* (2004), during sourdough fermentation higher amounts of folates can be produced than required to be consumed by the LAB. Our results indicated that higher levels of folates were obtained when fermenting the multigrain flour suspensions with SC2, followed by sourdough samples prepared with SC3 and SC1 (Figure 5).

In fact, when comparing the folates levels of sourdoughs fermented with LAB and sourdoughs obtained through spontaneous fermentation (controls), one can see that the sourdough prepared with SC1 had lower folate amounts in respect to the corresponding control samples (Figure 5). Most probably, the strains from SC1 consume more folates than can produce during sourdough fermentation. Moreover, the intracellular accumulation of the synthesized folate should be considered (Hugenholtz *et al.*, 2002). For instance, Sybesma *et al.* (2003) reported that *L. lactis* strain MG1363 is able to intracellularly accumulate 90% of the folate, releasing only small amounts into the fermentation medium.

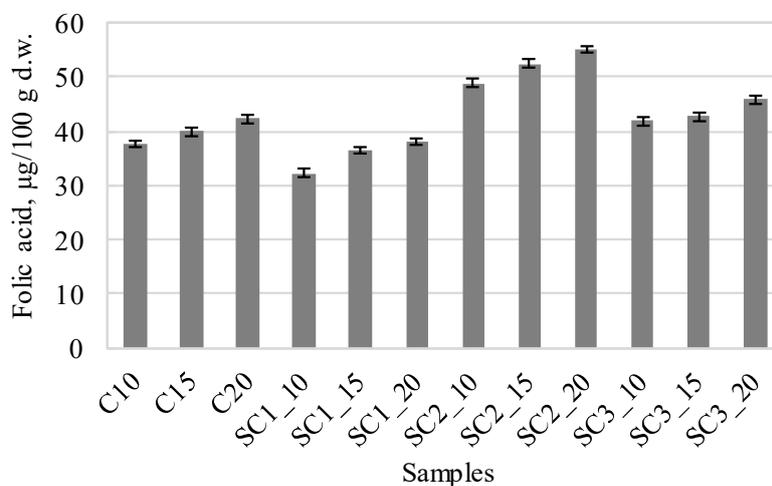


Figure 5. Folic acid content of the sourdoughs prepared without starter culture (C) and sourdoughs fermented with starter culture (SC)

Conclusions

The amounts of folate, phenolic acid and antioxidant activity of the multigrain flours, brans and shorts increased with the levels of rye and hulled oat in the

multigrain blends. The same trend was registered for TPC and DPPH-RSA in the case of multigrain flour, bran and short fractions. Shorts resulted through milling the multigrain blends with 10 and 15% rye and hulled oat had the highest contents of TPC among the obtained mill streams, while in case of multigrain blends with 20% rye and hulled oat, the highest TPC value was registered in bran. Regardless of the investigated multigrain blend, higher DPPH-RSA values were found for shorts than from brans. Finally, higher levels of folates were registered in brans and shorts, the amounts being about 1.3-1.4 and 1-1.3 times higher compared to the flours.

The sourdough fermentation induced the increase of TPC and DPPH-RSA compared to the flours. The LAB species from starter cultures impacted the accumulation of bioactive compounds in sourdoughs. The LAB metabolic end products accumulated in the sourdough increased the levels of easily extractible bioactive compounds. The accumulation of folates in the sourdough samples varied with the bacterial strains from the starter culture, suggesting that the LAB alike consume and produce folates.

Acknowledgments

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-BG-2016-0143, within PNCDI III.

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