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Wheat sprout flour as an attractive substrate for the producing probiotic fermented beverages: process development and product characterisation

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RESEARCH ARTICLE

Abstract

In this study, the fermented beverages based on wheat sprout flours (WSFs, 5, 10 and 15%) obtained from two varieties (NAVID and DEIM) using lactic probiotic bacteria of *Lactobacillus plantarum* and *Lactobacillus paracasei* were produced and characterised. Results revealed that the *L. paracasei* had a shorter dilation phase and a faster growth rate than *L. plantarum* especially in 5 and 15% WSF suspensions. The pH reduction was in accordance with the growth rate of two studied lactic bacteria. The fructose, glucose and maltose sugars were respectively consumed during the fermentation process. Lactic acid as the main organic acid at the process end was maximised in 15% WSF suspensions (0.70 g/100 ml). However, citric acid had the lowest changes during the process period. The 2,2'-diphenyl-1-picrylhydrazyl antioxidant activities of all beverages were significantly enhanced by the increasing fermentation time. Nevertheless, the lowest EC_{50} was found for 10% suspensions of DEIM-WSF fermented with *L. paracasei* starter. The results also showed that *L. paracasei* and DEIM sprout flour were the best strain and substrate, respectively, for production of functional fermented beverages in an industrial scale.

Keywords: wheat sprout, fermented beverage, fermentation process, lactic acid bacteria, antioxidant activity

1. Introduction

Cereals are cultivated over 70% of the total world harvested area and are contributed over 50% of world food production. The intact seeds of cereals are the sources of essential nutrients such as proteins, dietary fibres, antioxidants and vitamins (especially E and B-group) (Charalampopoulos *et al.*, 2002). Soluble wheat fibres particularly β-glucans which can reduce blood cholesterol, while insoluble fibres can short the transit time through the intestinal tract and decrease the contact between carcinogens and the epithelial cells in the colon (Sovrani *et al.*, 2012).

The presence of bioactive components (tocopherol, phytosterols, lignans, phenolic acid and folans) in cereals led to increase the antioxidant properties of products developed from these seeds. Furthermore, these seeds can be used in clinical application such as lowering the risk of cancer disease, decreasing the level of blood cholesterol and decreasing the rate of heart and cardiovascular disease (Hubner and Arendt, 2013; Rahaie *et al.*, 2012). Nevertheless, the nutritional quality of some cereals and the sensory properties of their products are sometimes inferior of poor compared to other staple-foods (Blandino *et al.*, 2003).

A variety of technologies (e.g. cooking, sprouting and milling) are used for cereal processing but fermentation still remains as the best choice for the improving nutritional, sensory and shelf-life properties (Blandino *et al.*, 2003). This is the main reason why a large proportion of cereals are processed into foods and beverages by fermentation prior to their consumption. In many African and Asian countries, cereal-based fermented beverages are consumed on a daily basis and at all ages (Nout, 2009). Since the excellent properties of wheat sprout and its use in all over the world, there is a great concern about application of wheat sprout in the production of fermented beverage (Katina *et al.*,

2007). The use of wheat germ to prepare this fermented beverage is due to fortify vitamin E, B-group and dietary fibres via fermentation process and thus improves its quality and nutritional characteristics (Sidhu and Kabir, 2007). Thus, the aim of this study was to develop and characterise the fermented beverage using two kinds of wheat sprout flour (WSF) by the cultivation of *Lactobacillus* strains of *Lactobacillus plantarum* and *Lactobacillus paracasei*.

2. Materials and methods

Preparation of wheat sprout flour

The certified wheat grains without any pests and diseases were purchased from collection centres wheat and flour mills. At this stage, five varieties of wheat were selected. The cleaned grains (100 g) rinsed for 20 h in running tap water at 28-30 °C. After the steeping, wheat grains were immersed in 2% sodium hypochlorite solution (Merck Chemical Co., Darmstadt, Germany) for 10 min and then rinsed 5 times with excess of water. The grains were germinated for 3 to 5 days at 28 °C and then dried in an oven at 50 °C for 24 h (Coda *et al.*, 2011). In this way, malting in the wheat secondary roots and buds is not isolated because of presence of beneficial bioactive compounds (Katina *et al.*, 2007). WSFs from two varieties of 'DEYM' and 'NAVID' were selected after their germination, drying and milling steps. They were selected according to the percentage of carbohydrates and proteins. Higher carbohydrate and protein less than 11.5 g/100 g were considered for the beverage production (Munoz-Insa *et al.*, 2013).

Preparation and activation of the bacterial strains

L. plantarum (20174DSMZ) and *L. paracasei* (20207DSMZ) were prepared from DSMZ Co. (Braunschweig, Germany). Activation with double passage of bacteria on sterile de Man, Rogosa and Sharp (MRS; Merck) was performed. These bacteria were cultivated in MRS broth medium until the late exponential growth phase was reached. Then, surface culture was carried out on solid MRS plates and incubated for 24-48 h at 37 °C. The cream-coloured colonies are represented as biologically active lactic bacteria.

Suspension preparation of WSF

For the preparation of suspensions containing 5, 10 and 15% WSF, respectively, 5, 10 and 15 g of WSF were mixed with 100 ml distilled water. They were then pasteurised in 63.3 °C for 30 min (Coda *et al.*, 2011).

Inoculation of the suspension with the activated bacteria

For preparation of an initial cell density of 10^6 colony forming units (cfu)/ml, the bacteria were added to 100 ml of WSF suspension in sterile condition. The mixture of suspension and bacterial strains were incubated at 30 °C until the pH value of the mixture decreased to 4. The growth curves of the bacterial strains in suspension were prepared by counting cfu's on MRS agar medium (Mousavi *et al.*, 2013).

Determination of pH and total titratable acidity

The values of pH were determined using a pH-meter (model 691; Metrohm, Herisau, Switzerland) equipped to a food penetration probe (ISIRI, 2011). The total titratable acidity (TTA) was determined by titrating the sample (10 g beverage homogenised with 90 ml distilled water) with 0.1 N NaOH solution (Merck) to get a pH of 8.3 (Coda *et al.*, 2011).

Identification and quantification of the sugar content

The method of Miguel *et al.* (2004) with minor modification was applied using a high-performance liquid chromatography (HPLC) system (K-2600; Knauer, Berlin, Germany). All the samples before of injection were filtered through a 0.2 µm nylon-syringe filter (model FNY-402-030; Jet Biofil, Kyoto, Japan). A column (Eurokat H 10 µm, 300×8 mm; Knauer) at 30 °C and a K-2310 RI detector were used to separate different sugars. Sulfuric acid 0.01 N as the mobile phase of was injected at a flow rate of 0.5 ml/min.

Identification and quantification of the organic acids content

An HPLC system (K-2600; Knauer) was used to analyse the main organic acids including lactic, acetic, citric and formic acids in WSF. The separation process of organic acids was carried out using an Ultrasep column (ES FS, 250×3 mm; Knauer) at 30 °C and a UV-visible K-2600 detector at 210 nm. The mobile phase of 2.25 mM sulphuric acid was injected with a flow rate of 0.3 ml/min under a linear gradient. As the sugar determination, all the samples before of injection were filtered through a 0.2 µm nylon-syringe filter (Jet Biofil). Finally, the amount of organic acids by comparing the standard curve and the obtained peaks were determined (Mousavi et al., 2013).

Antioxidant activity measurement

The antioxidant activities of fermented beverages were determined using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma Chemical Co., St. Louis, MO, USA) as previously reported by Nithiyanantham *et al.* (2012).

For the preparing fresh methanol solution of DPPH (0.06 mM) 0.0025 g DPPH powder was dissolved in 100 ml distilled water. A serial dilution of the samples (0.1-1.0 w/w) was also prepared. 3 ml of DPPH solution was added to 1 ml of each sample in the tubes and the obtained mixtures were well shaken in a vortex with 2,500 rpm for 1 min and then placed under dark conditions for 30-60 min. The

absorbance decrease at 517 nm was determined with a UV-visible spectrophotometer (BioQuest CE 2502; Cecil Instruments Ltd., Cambridge, UK). Absorbance of DPPH radical without the sample containing antioxidant was used as control. The sample amount necessary to decrease 50% the free radicals (EC_{50}) was graphically calculated. Inhibition percentage of the DPPH radical was also calculated from the following equation:

$$\text{Inhibition percentage} = \frac{C_1 - C_2}{C_1} \times 100 \quad (1)$$

Where C_1 and C_2 are respectively absorbance of control sample and absorbance of a tested sample at the reaction end.

Statistical analysis

All analytical experiments for the different samples in triplicate were carried out and the results presented as a mean of the three values with the standard deviation. Analysis of variance (ANOVA) procedure followed by Duncan's test using SPSS 13 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference (P<0.05) between treatment means.

3. Results and discussion

Growth rate of the lactic bacteria strains

Growth curves of the lactic bacterial strains in the concentration range of 5 to 15% microbial suspension are presented in Figure 1. As considered in this figure, the growth rate of *L. paracasei* was faster than *L. plantarum* in WSFs of both DEIM and NAVID varieties (P<0.05). *L. paracasei* growth was initiated in logarithmic phase without dilation and then entered in the phase death. However, the growth of *L. plantarum* with dilation was initiated in the logarithmic phase on both WSFs prepared from DEIM and NAVID varieties. Moreover, a more dilation was observed for the obtained substrate from NAVID variety. A 6-h lag phase was found at suspensions containing 10% WSF concentration (Figure 2). But at the suspensions containing 15% WSFs, the dilation phase for *L. plantarum* was longer than another strain. Moreover, *L. paracasei* at the same concentration entered to its logarithmic phase faster than another strain (Figure 3).

In general, for the beverage production form WSF, *L. paracasei* in comparison to *L. plantarum* had a faster growth rate at suspension concentrations of 5 and 15% WSF. But, no significant difference in growth rate of two studied lactic bacteria at suspension concentration of 10% WSF was detected. The best concentration of WSF suspension was 5% especially for *L. paracasei. L. plantarum* has an important role in the production of fermented foods and beverages because shelf life of these products with releasing the antimicrobial agents by *L. plantarum* can be significantly increased (Todorov and De Melo Franco, 2010). Ramos *et al.* (2010) by studying the production of a Brazilian beverage named 'Cauim' containing cassava, rice and millet found that lactic bacteria of *L. plantarum*, *L. fermentum* and *L. paracasei* were dominant at the fermentation end.

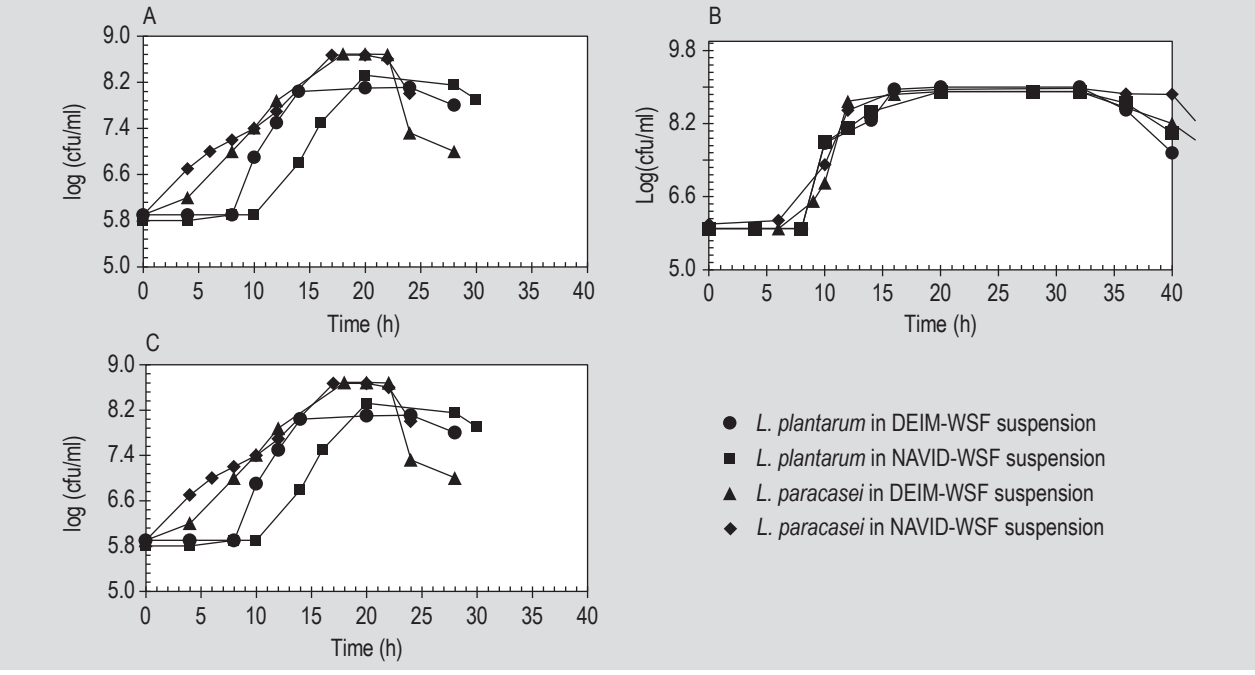

Figure 1. Growth curves of *Lactobacillus plantarum* and *Lactobacillus paracasei* strains on wheat sprout flours (WSF) suspensions at different concentrations of (A) 5, (B) 10 and (C) 15% from the initial of the fermentation process to the bacterial death time.

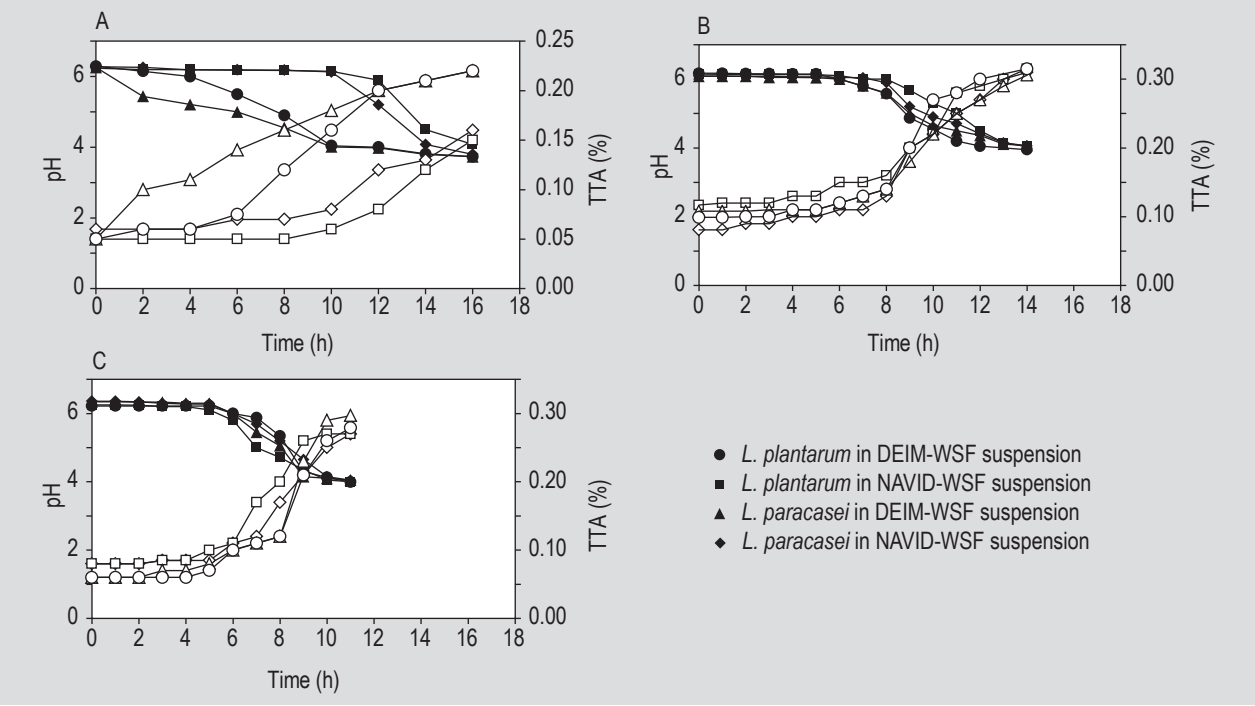

Figure 2. Changes in the pH (dark symbol) and total titratable acidity (TTA; white symbol) values of beverages fermented by *Lactobacillus plantarum* and *Lactobacillus paracasei* strains at the different concentrations of (A) 5, (B) 10 and (C) 15% wheat sprout flours (WSF)-suspensions.

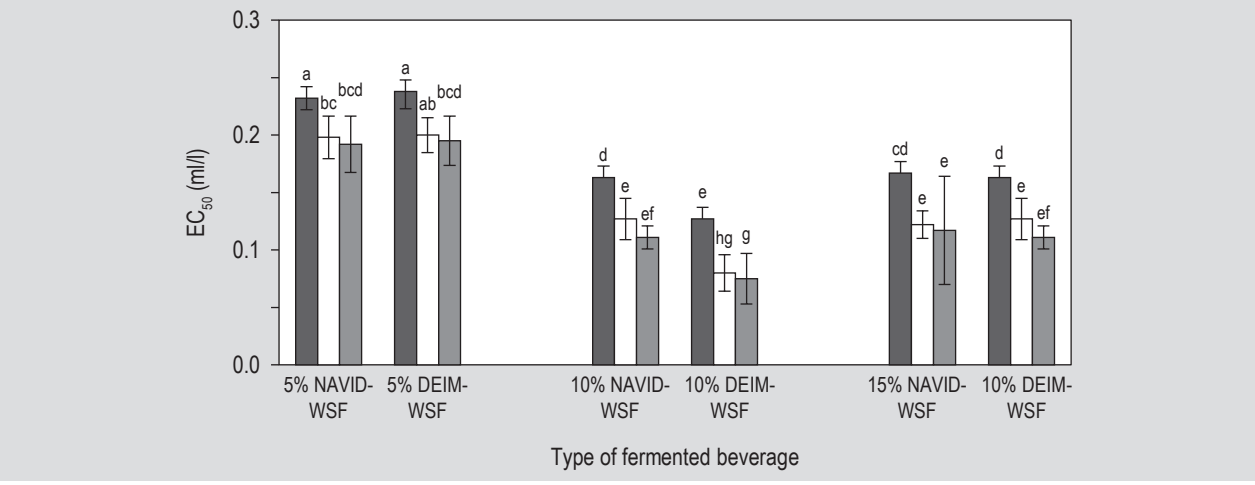

Figure 3. Changes in the antioxidant activities of beverages fermented by *Lactobacillus plantarum* (white column) and *Lactobacillus paracasei* (grey symbol) compared to the control (dark column) from the process initial to final time. Values in the same column followed by different letters are significantly different (*P*<0.05)

Changes of the pH and acidity during lactic fermentation

The changes in pH and TTA values in 5, 10 and 15% suspensions of WSFs after the adding lactic bacteria can be seen in Figure 2. According to Figure 2, the pH values decreased from 6.5 to 4.0 during fermentation process. The pH decrease rate in fermented beverage is more appropriate for *L. paracasei* activity in two types of WSFs. There was a dilation in decreasing pH of the beverages fermented with *L. plantarum* in both types of WSFs. However, this dilation was more significant in WSF prepared from NAVID variety ($P<0.05$).

The initial pH in 10% suspensions of WSFs was 6.2. This value was stable during the first 5 h of the fermentation process. After this period, the pH of beverages was decreased to 4.0 due to the activation of lactic bacteria and fermentation process with the initiation of logarithmic

phase. The initial pH in 15% suspensions of WSFs was 6.2. It was stable during the first 6 and 8 h, respectively, for fermentation process by *L. paracasei* and *L. plantarum*. The pH of beverages after this period with the initiation of logarithmic phase due to the activation of lactic bacteria was decreased to 4.0. The decreasing rate in pH value of beverages to an appropriate level (pH=4) for *L. paracasei* in 5 and 15% suspensions of WSFs was faster than *L. plantarum* (P<0.05). But, there were no significant difference between two studied lactic bacteria in the decreasing pH value in 10% suspensions of WSFs.

Muyanja *et al.* (2012) showed a negligible reduction (0.13 unit) in pH value for naturally fermented beverage called 'Bushera' using lactic starters during the first 4 h of fermentation time. This was followed by a more rapid pH drop to approximately pH 4.0 after 24 h, which is comparable to that of the starters used in this study. They found the fastest drop in pH level during first 24 h, thereafter pH reduction was slowed and stabilised between pH 3.5 and 4.0 depending on the starter (Muyanja *et al.*, 2012).

Changes in the sugar content

Changes in the sugar content of produced beverages are given in Table 1. The sugar content of the samples with the different suspension concentrations at the initial fermentation process was as follows: fructose > glucose > maltose. Moreover, the reduction rate of sugar content at the end of the fermentation process was as follows: fructose > glucose > maltose.

During the fermentation of *L. paracasei* on 5% suspension of DEIM-WSF (pad5), the sugars consumption was significantly (P<0.05) more than the *L. plantarum* fermentation on 5% suspension of DEIM-WSF (pld5). The reduction rate of sugars during the *L. plantarum* fermentation was more than the *L. paracasei* fermentation on 10% suspension of DEIM-WSF (pad10). The maltose reduction rate at 15% DEIM-WSF suspension by *L. plantarum* (pld15) was significantly more than *L. paracasei* at the same substrate (pan15). The maltose reduction rate by *L. paracasei* at a suspension containing 10% NAVID-WSF (pan10) was significantly more than *L. plantarum* at the same substrate (pln10). Moreover, the contents of glucose and fructose in WSF suspensions were completely used in both suspensions of the studied lactic bacteria. The reduction rate of maltose by *L. plantarum* (pln15) was more than the *L. paracasei* (pan15) on 15% NAVID-WSF. Moreover, the glucose and fructose contents of suspensions were completely used in both suspensions of the lactic bacteria.

In general, the sugars consumption of each lactic bacterium is depended on substrate and the period of fermentation process (Hou and Chou, 2000). *L. plantarum* compared to *L. paracasei* in the Bushera production showed more activity for consumption of maltose, glucose and fructose (Muyanja *et al.*, 2012). The main sugars in the functional emmer beverage were maltose, glucose and fructose (Coda *et al.*, 2011). The overall consumption of sugars by these lactic bacteria during fermentation is dependent on the type of raw material and fermentation time (Hou and Chou 2000).

Table 1. Changes in sugar content (mean ± standard deviation) of the beverages in fermentation process using two studied lactic bacteria from the process initial (t_0) to the final time (t_f).[1]

Beverage type[2]	Sugars (g/l) at t_0			Sugars (g/l) at t_f		
	Maltose	Glucose	Fructose	Maltose	Glucose	Fructose
Pld 5	7.18±0.25^{d}	2.71±0.10^{d}	0.98±0.17^{d}	5.05±0.22^{c}	1.12±0.16^{b}	0.20±0.15^{b}
Pad 5	8.15±0.22^{c}	1.24±0.15^{e}	0.83±0.15^{e}	3.03±0.28^{e}	0.45±0.14^{c}	–
Pln 5	6.59±0.20^{e}	1.31±0.13^{e}	1.34±0.10^{c}	4.95±0.21^{c}	0.28±0.15^{c}	0.20±0.13^{b}
Pan 5	5.20±0.18^{f}	1.55±0.12^{e}	1.10±0.10^{d}	3.31±0.22^{e}	0.35±0.10^{c}	0.18±0.16^{b}
Pld 10	7.70±0.12^{d}	2.82±0.11^{d}	1.35±0.13^{c}	3.95±0.25^{d}	–	–
Pad 10	11.91±0.20^{a}	5.09±0.23^{a}	1.93±0.10^{b}	9.05±0.25^{a}	2.81±0.16^{a}	0.85±0.13^{a}
Pln 10	6.69±0.21^{e}	2.62±0.14^{d}	1.11±0.10^{d}	6.62±0.26^{b}	–	–
Pan 10	9.87±0.23^{b}	3.85±0.15^{c}	1.57±0.15^{c}	7.40±0.27^{b}	–	–
Pld 15	8.30±0.25^{c}	4.40±0.11^{b}	2.00±0.11^{b}	4.10±0.29^{d}	–	–
Pad 15	8.90±0.10b^{c}	5.30±0.10^{a}	2.30±0.12^{a}	5.10±0.29^{c}	–	–
Pln 15	9.90±0.23^{b}	4.36±0.20^{b}	2.30±0.12^{a}	6.80±0.20^{b}	–	–
Pan 15	11.90±0.21^{a}	4.21±0.16^{b}	1.88±0.19^{b}	5.63±0.21^{c}	–	–

[1] Values in the same column followed by different letters are significantly different (P<0.05).

[2] Pa = *Lactobacillus paracasei*; Pl = *Lactobacillus plantarum*; n = NAVID wheat variety; d = DEIM wheat variety; 5, 10 and 15 = percentage of WSF substrate used in the fermentation medium.

Changes in the organic acid content

As considered in Table 2, the most important organic acid in all the samples was lactic acid. This acid form the initial of the fermentation process to its end was increasingly produced with the lactic bacteria. The highest rate of lactic acid production was for both varieties of *L. plantarum* in 15% WSF suspension as this rate respectively was increased from 0.09 g/10 to 0.70 g/100 ml and from 0.05 to 0.70 g/100 ml.

The main organic acid in the fermented beverages is lactic acid which can be used as flavouring, acidification and reservoir agent in these beverages (Liu, 2003). Furthermore, pyruvic acid (substrate of lactate) can be applied for production of other important compounds in fermented beverages (Liu, 2003). It was also demonstrated that the major organic acids produced by the cereals fermentation are lactate and acetate (Bley *et al.*, 2004).

The change in antioxidant activities of beverages during the fermentation process using two lactic bacteria is presented in Figure 3. As can be seen in this figure, EC_{50} values of the beverages with 5, 10 and 15% WSF suspensions significantly were lower than the control (P<0.05). Figure 3 showed that an increase in antioxidant content of the beverages at the end of fermentation process. There is no difference in antioxidant content in the beverages made from 5 and 15% WSF suspensions in both the lactic bacteria. The antioxidant contents of the DEIM variety of *L. paracasei* and *L. plantarum* in a 10% WSF suspension were significantly more than of the NAVID variety (P<0.05). These results were in an agreement with the obtained findings by Mousavi *et al.* (2013). These researchers found that the antioxidant capacities of pomegranate juice based-fermented beverages at the end of fermentation in comparison with the control were significantly increased (Mousavi *et al.*, 2013). It was revealed that the consumption of foods and beverages with high content of polyphenols and antioxidants, led to reduce oxidative stress related to the disease. (Gharibzahedi *et al.*, 2013; Viuda-Martos *et al.*, 2011; Zujko and Witkowska, 2013).

4. Conclusions

In this study, production and characterisation of the fermented beverages from WSF using two lactic bacteria of *L. plantarum* and *L. paracasei* were studied. The results showed that *L. paracasei* had faster growth rate on 5% WSF suspension without dilation phase compared to another bacterium. The WSF and bacterium type had no significant difference in the cell growth at suspension concentration of 10% WSF. However, *L. plantarum* in 15% WSF suspensions of had shorter dilation phase compared to *L. paracasei*.

The reduction in pH values is in accordance with growth of the lactic bacteria. Maltose, glucose and fructose, respectively, had the highest consumption rate. The antioxidant activities of beverages at the end of fermentation process were significantly increased in all the samples. The highest antioxidant content was observed for 10% WSF suspensions obtained from DEIM variety. The

Table 2. Changes in organic acid content (mean ± standard deviation) of the beverages in fermentation process using two studied lactic bacteria from the process initial (t_0) to final time (t_f).[1]

Beverage type[2]	Organic acids (g/100 ml) at t_0				Organic acids (g/100 ml) at t_f			
	Lactic	Formic	Acetic	Citric	Lactic	Formic	Acetic	Citric
Pld5	–	0.05±0.01^{b}	0.002±0.001^{d}	0.008±0.002^{c}	0.26±0.02^{d}	0.15±0.03^{b}	0.10±0.02^{c}	0.09±0.02^{a}
Pln5	–	0.02±0.01^{b}	0.019±0.002^{c}	–	0.22±0.01^{d}	0.10±0.02^{c}	0.07±0.01^{d}	–
Pad5	–	0.09±0.02^{a}	0.004±0.002^{d}	0.009±0.001^{c}	0.28±0.01^{d}	0.16±0.02^{b}	0.02±0.00^{d}	0.02±0.00^{b}
Pan5	–	0.07±0.01^{a}	0.002±0.002^{d}	0.009±0.001^{c}	0.28±0.01^{d}	0.10±0.02^{c}	0.05±0.01^{d}	0.05±0.01^{b}
Pld10	–	0.03±0.01^{b}	0.013±0.002^{c}	0.015±0.002^{b}	0.50±0.02^{b}	0.07±0.01^{c}	0.03±0.00^{d}	0.01±0.00^{b}
Pln10	–	0.02±0.01^{b}	0.050±0.020^{b}	0.011±0.002^{b}	0.39±0.01^{c}	0.09±0.02^{c}	0.12±0.01^{c}	0.02±0.00^{b}
Pad10	0.04±0.01^{b}	0.02±0.01^{b}	0.090±0.030^{b}	0.080±0.030^{a}	0.52±0.01^{b}	0.14±0.02^{b}	0.11±0.01^{c}	0.11±0.01^{a}
Pan10	0.26±0.01^{a}	0.08±0.02^{a}	0.290±0.030^{a}	0.025±0.002^{b}	0.63±0.02^{a}	0.09±0.02^{c}	0.42±0.00^{a}	0.03±0.01^{b}
Pld15	0.09±0.01^{b}	0.04±0.01^{b}	0.040±0.010^{b}	0.003±0.001^{c}	0.70±0.01^{a}	0.10±0.01^{c}	0.30±0.02^{b}	–
Pln15	0.05±0.02^{b}	0.07±0.02^{a}	0.020±0.010^{b}	0.010±0.008^{c}	0.70±0.01^{a}	0.20±0.02^{a}	0.09±0.01^{c}	0.03±0.01^{b}
Pad15	–	0.05±0.01^{b}	0.010±0.001^{c}	0.020±0.010^{b}	0.45±0.01^{b}	0.17±0.01^{a}	0.20±0.02^{c}	0.05±0.01^{b}
Pan15	0.10±0.02^{b}	0.05±0.01^{b}	0.070±0.021^{b}	0.010±0.008^{c}	0.60±0.02^{a}	0.20±0.02^{a}	0.22±0.01^{c}	0.03±0.01^{b}

[1] Values in the same column followed by different letters are significantly different (P<0.05).

[2] Pa = *Lactobacillus paracasei*; Pl = *Lactobacillus plantarum*; n = NAVID wheat variety; d = DEIM wheat variety; 5, 10 and 15 = percentage of WSF substrate used in the fermentation medium.

reduction period of pH to 4 in 10% WSF suspensions was less than the other samples which is economically acceptable. *L. paracasei*, because of faster growth rate and other investigated properties, was more preferable for the production of functional fermented beverages. Owing to the lower cost of WSF prepared form DEIM variety and higher antioxidant activity of its fermented beverage, this kind of wheat is more profitable for the application in bio and food industries.

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