MILLET GRAINS AS AN IMMOBILIZING MATRIX TO CARRY PROBIOTICS IN DRY FERMENTED SAUSAGES

ABSTRACT

The aim of this study was to assess the effect of immobilized probiotic *Lp. plantarum* in millet grains on the physical chemical and microbiological profiles of dry-fermented sausage during ripening and storage. Fermented sausages were distributed in 4 treatments: fermented sausages containing free cells of *Lp. plantarum* (FC), free cells of *Lp. plantarum* + 4% sterile millet grains (NI4%), 2% immobilized *Lp. plantarum* in millet (I2%), 4% immobilized *Lp. plantarum* in millet (I4%). Sausages were evaluated during ripening (0, 2, 7, 14 and 21 days), and storage (41, 61, 81, 101, 121 days) for pH, water activity, instrumental color, texture profile, starter and probiotic culture counts in sausages and during *in vitro* simulated oro-gastro-intestinal digestion. Physicochemical characteristics (pH and aw) ranged within the levels usually observed in fermented sausages. Adhesiveness, gumminess, and chewiness of dry-fermented sausages were affected by immobilization ($P < 0.05$), however treatments showed similar profile during most of the period evaluated. Immobilization (I2% and I 4%) significantly improved the viability of *Lp. plantarum* during simulated digestion. Free and immobilized cells were found to be viable up to 121 days of storage (>7 log CFU g^{-1}) at room temperature, however immobilized *Lp. plantarum* showed higher counts than the free cells ($P<0.05$). The immobilization did not impact the physical chemical characteristics of the product. Millet grains proved to be a suitable matrix for the immobilization of *Lp. plantarum* in dry fermented sausages and maintained cells viability at levels above 7 log CFU g^{-1} in sausages for a long-term period (> 3 months) under non-refrigerated storage conditions.

INTRODUCTION

In recent years, different strategies have been proposed to develop healthy meat products (Heck et al., 2017). The functional value improvement can be achieved by adding functional ingredients such as vegetable proteins, dietary fibers, herbs, spices, and probiotic bacteria incorporated into meat products during processing (Zhang et al., 2010). The use of probiotic strains in the fermented meat has gained space because of the numerous benefits provided by the consume of these microorganisms. In addition, fermented sausages are ready for consumption, meeting the demand for practicality and convenience (Bis-Souza et al., 2020). Fermented sausage that are consumed without cooking are considered efficient vehicles for probiotic bacteria, since non-heating favors the maintenance of the cellular viability of microorganisms (Pasqualin Cavalheiro et al., 2015).

In meat fermentation, lactic acid bacteria (LAB), such as *Lactiplantibacillus plantarum*, promote rapid acidification of the batter leading to lower pH values with improving the microbial stability of products by inhibiting the activity of pathogens. Starter LAB bring about the physicochemical and biochemical changes to attain the unique sensory features of ripe products during the maturation and fermentation (Vuyst et al., 2008). Moreover, they are able to tolerate simulated gastrointestinal conditions and survive to the matrix conditions, e.g., presence curing salts and low pH and water activity (Fenster et al., 2019).

Probiotics, particularly when included in dietary supplements, are commonly transported and stored at room temperatures and humidity. This may lead to loss of viability as compared to refrigerated/frozen storage and handling (Sreeja & Prajapati, 2013). Moreover, the production of foods with probiotic claims is a challenge, especially due to difficulties of survival and maintenance of the probiotic cells added to the foods under processing, storage, distribution, and consumption conditions (Min et al., 2017). Therefore, immobilization techniques are generally applied to maintain the activity and the functionality of probiotic cells when incorporated into food matrices, since extreme conditions are often employed during food processing and storage (Mitropoulou et al., 2013). Several types of grains have been reported as supports, of which some examples are wheat and barley (Kandylis et al., 2012; Sidira et al., 2015). However, there are still no reports in the literature of millet grain used as a support for the immobilization of *Lp. plantarum* and its application in dry fermented sausages.

OBJECTIVE

The aim of the present study was to evaluate the effect of immobilized probiotic *Lp. plantarum* cells in millet grains on the physical chemical and microbiological profiles of dry-fermented sausage during ripening and storage.

RESULTS AND DICUSSION

The pH was affected by time (ripening and storage) and treatment (Figure 1). During ripening, pH decreased due to the production of organic acids such as lactic acid and acetic acid by LAB (Ammor & Mayo, 2007). At the end of the ripening (21R), FC showed the lowest pH value (6.22) whereas NI 4% and I 4% showed the highest values and, were statistically equal. There was no significant difference in pH among treatments at the end of the storage time (121 S). The aw was only affected by time. The aw of the sausages decreased (P< 0.05) during ripening, from an initial value of 0.996 to 0.888, showing no differences among treatments (Fig 1).

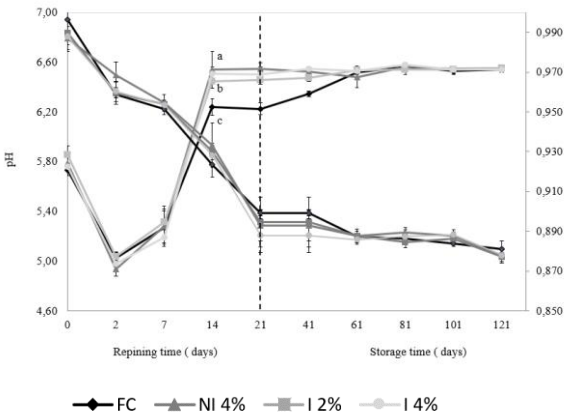

Fig. 1: Counts of *Lp. plantarum* in dry-fermented sausages after ripening (21 days) during simulated *in vitro* digestion and (b) Counts of *Lp. plantarum in* dry-fermented sausages after storage time (121 days) simulated *in vitro* digestion. Different letters indicate statistical differences ($P < 0.05$); FC = free cell; NI 4 % = free cell + 4% sterile millet grains); I 2% = 2 % immobilized *Lp. plantarum*; I 4% = 4 % immobilized *Lp. plantarum*

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Time and treatments influenced the probiotic count (Fig. A). At 121 S, fermented sausages with probiotic immobilized maintained the counts at high levels (> 8 log CFU g^{-1}), demonstrating that there was a protection of *Lp. plantarum* by the grains during storage. Immobilized *Lp. plantarum* were detected in sausages until the end of the long-term storage at levels higher than the required for conferring a probiotic effect.

The results of *Lp. plantarum* count during simulated digestion after ripening (21 R) and after storage (121 S) are shown in Figure 1-B1-B2. In both conditions, the viability was affected by time and treatments. At the 21 R, FC treatment showed the highest cell reduction (0.76 log) on the probiotic count during digestion. Moreover, at 121 S immobilized cells, I 2% and I 4%, presented higher count and lower cell reduction than the non-immobilized cells after simulated digestion, 0.15 and 0.16 log, respectively

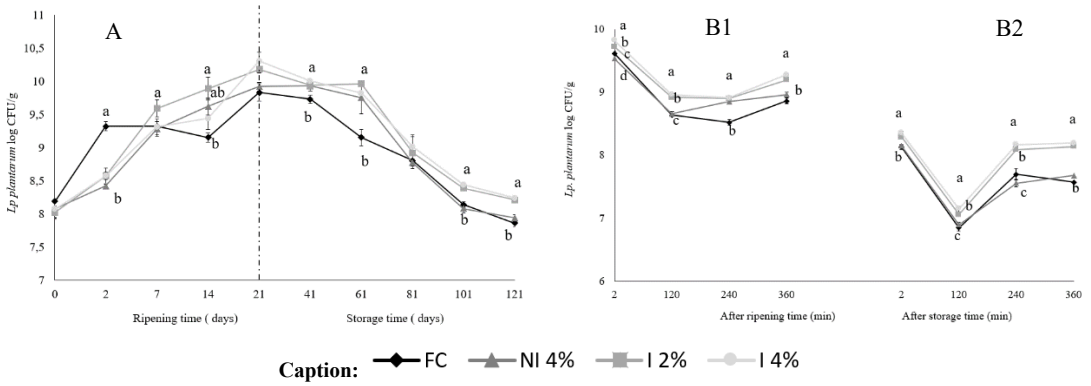

Fig. 1 A-B: (A) Counts of *Lp. plantarum* in dry-fermented sausages during ripening (21 days) and storage (121 days). (B1) Counts of *Lp. plantarum* in dry-fermented sausages after ripening (21 days) during simulated *in vitro* digestion and (B2) Counts of *Lp. plantarum in* dry-fermented sausages after storage time (121 days) simulated *in vitro* digestion. Different letters indicate statistical differences ($P < 0.05$); FC = free cell; NI 4 % = free cell + 4% sterile millet grains; I 2% = 2 % immobilized *Lp. plantarum*; I 4% = 4 % immobilized *Lp. plantarum*.

Regarding texture parameters, adhesiveness, gumminess, and chewiness of dry-fermented sausages were affected by both time and treatment ($P<0.05$), whereas hardness, springiness and cohesiveness were only affected by time (Table 2). Adhesiveness had a significant drop in 7 R, however after this period, it increased in all treatments which remained stable until the end of storage. All treatments showed increase in hardness and decrease in springiness and cohesiveness with the extension of the ripening. Hardness remained relatively constant, and springiness and cohesiveness increased over storage time. Gumminess and chewiness increased over ripening and during storage. According to Bolzan and Pereira (2017), gumminess is a secondary parameter, associated with firmness and cohesiveness. At the end of the ripening, treatments showed difference only for cohesiveness for which I2% showed higher value than the control. At the end of the storage, difference was for only for gumminess and chewiness, for which NI 4% showed higher value than control. Despite these few differences, treatments showed similar profile indicating that immobilization did not affect the texture profile of the fermented sausages.

Table 2: Effect of millet grains addition and *Lp. plantarum* immobilization on the textural properties of dry fermented sausages.

		2 R	7 R	21 R	41 S	81 S	121 S
Hardness (N)	FC	58.39±12.36^{aC}	49.54±10.56^{aC}	197.57±7.13^{aAB}	180.75±13.83^{aB}	178.77±20.16^{aB}	207.48±10.44^{abA}
	NI 4%	57.52±6.64^{aB}	42.99±19.77^{aB}	205.02 ± 25.59^{aA}	193.63±15.25^{aA}	203.1±19.61^{aA}	188.63±21.75^{bA}
	I 2%	61.61±4.93^{aB}	51.89±5.67^{aB}	212.84±67.47^{aA}	204.03±26.02^{aA}	186.35±15.33^{aA}	209.72±14.55^{abA}
	I 4%	54.91±4.25^{aC}	59.16±8.75^{aC}	204.38±31.04^{aAB}	187.58±28.08^{aB}	190.62±18.84^{aB}	236.65±20.49^{aA}
	FC	-1.07±1.23^{aA}	-4.27±0.33^{bB}	-0.14±0.11^{aA}	-0.69±0.25^{bA}	-0.13±0.06^{aA}	-0.18±0.09^{bA}

Adhesiveness (N.cm)	NI 4%	-0.94±0.41^{aB}	-2.70±1.23^{aB}	-0.07±0.07^{aA}	-0.10±0.11^{aA}	-0.14±0.00^{aA}	-0.04±0.03^{aA}
	I 2%	-1.02±0.41^{aB}	-3.89±0.36^{bC}	-0.07±0.02^{aA}	-0.11±0.06^{aA}	-0.15±0.06^{aA}	-0.08±0.05^{abA}
	I 4%	-0.66±0.31^{aB}	-4.22±0.44^{bC}	-0.30±0.26^{aAB}	-0.27±0.09^{aAB}	-0.09±0.06^{aA}	-0.07±0.05^{aA}
Springiness (cm)	FC	0.84±0.03^{aA}	0.73±0.06^{aB}	0.59±0.03^{aD}	0.63±0.02^{aCD}	0.72±0.05^{aB}	0.68±0.02^{aBC}
	NI 4%	0.80±0.03^{aA}	0.79±0.06^{aAB}	0.59±0.04^{aD}	0.63±0.04^{aCD}	0.68±0.09^{aBC}	0.69±0.03^{aC}
	I 2%	0.85±0.03^{aA}	0.74±0.02^{abB}	0.57±0.05^{aD}	0.67±0.05^{aC}	0.70±0.03^{aBC}	0.70±0.03^{aBC}
	I 4%	0.80±0.02^{bA}	0.71±0.02^{bB}	0.59±0.02^{aC}	0.62±0.04^{aC}	0.70±0.04^{aB}	0.71±0.01^{aB}
Cohesiveness ratio	FC	0.68±0.01^{aA}	0.64±0.01^{aB}	0.42±0.01^{bD}	0.51±0.01^{aC}	0.54±0.03^{aC}	0.54±0.02^{aC}
	NI 4%	0.66±0.02^{bA}	0.64±0.01^{aA}	0.42±0.03^{bD}	0.51±0.04^{aC}	0.55±0.01^{aBC}	0.57±0.01^{aB}
	I 2%	0.67±0.01^{abA}	0.64±0.02^{aA}	0.47±0.02^{aD}	0.51±0.03^{aC}	0.58±0.04^{aB}	0.55±0.02^{aBC}
	I 4%	0.65±0.02^{bA}	0.62±0.02^{bB}	0.41±0.02^{bE}	0.50±0.03^{aD}	0.56±0.01^{abC}	0.56±0.02^{aC}
Gumminess (N.cm^2)	FC	39.96±8.41^{aC}	31.37±6.14^{aC}	83.46±5.25^{aB}	92.61±5.95^{aB}	96.55±13.94^{aAB}	112.46±7.92^{bA}
	NI 4%	37.82±4.52^{aC}	27.35±12.56^{aC}	85.32±5.73^{aB}	99.45±9.66^{abAB}	112.13±13.65^{aA}	106.72±11.7^{bA}
	I 2%	41.57±3.69^{aB}	33.28±3.52^{aB}	98.7±28.65^{aA}	104.41±7.86^{aA}	107.00±3.87^{aA}	115.51±9.67^{abA}
	I 4%	35.84±2.04^{aD}	36.42±5.32^{aD}	82.96±9.95^{aC}	93.41±9.38^{abBC}	106.90±9.43^{aB}	133.18±16.15^{aA}
Chewiness (N/cm^2)	FC	33.46±7.52^{abD}	23.09±6.49^{aD}	48.97±4.42^{aC}	58.00±4.87^{aBC}	69.24±12.09^{aAB}	76.66±6.16^{bA}
	NI 4%	30.34±4.32^{abC}	21.27±10.02^{aC}	50.29±5.84^{aB}	62.90±6.47^{aAB}	76.29±13.41^{aA}	73.35±9.67^{bA}
	I 2%	35.49±3.31^{aD}	24.83±3.10^{aE}	55.31±13.89^{aC}	69.85±8.64^{aAB}	75.13±3.98^{aA}	80.39±8.71^{abA}
	I 4%	28.57±1.50^{aD}	26.00±3.94^{aD}	49.13±5.61^{aC}	57.75±6.33^{aC}	74.98±6.21^{aB}	94.18±10.41^{aA}

^{a,b,c,d} - different lowercase letters in columns indicate significant difference among treatments ($P < 0.05$); $^{A, B, C, ...}$ – different uppercase letters in rows indicate significant difference among times ($P < 0.05$); SEM - standard error of the mean. FC= free cell; NI 4 %=free cell + 4% sterile millet grains; I 2% = 2 % immobilized *Lp. plantarum*; I 4% = 4 % immobilized *Lp. plantarum.*

CONCLUSION

Millet grains proved to be a suitable matrix for the immobilization of *Lp. plantarum* in dry fermented sausages. The immobilization did not impact the physical chemical characteristics of the product. The technique protected probiotic against the adverse conditions of simulated gastrointestinal digestion and maintained cells viability at levels above 7 log CFU g^{-1} in sausages for a long-term period (> 3 months) under non-refrigerated conditions. Considering the recommended probiotic daily intake (~9 log CFU per serving), the beneficial effect could be achieved with the ingestion of 10 g (1/2 slice) of fermented sausage per day, which is feasible and compatible with a nutritionally balanced diet.

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