冷冻和冻干处理对酸面团中乳酸菌活力的影响

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摘要 [目的]研究冷冻及冻干处理对酸面团中乳酸菌活力的影响。[方法]选取植物乳酸菌 M616 为发酵菌株制作酸面团,分别进行冷冻(-80 ℃)和冻干预处理,-20 ℃冻藏 30 d,考察冻藏期内乳酸菌的存活率、产酸能力及酸面团的 pH、总酸度(TTA)的变化。[结果]随着冻藏时间的延长,冷冻和冻干酸面团中乳酸菌的存活率均显著下降,但相同冻藏时间下,冷冻酸面团中的乳酸菌存活率显著高于冻干酸面团中的乳酸菌存活率;冻藏第 0、7 天时,冷冻和冻干酸面团中乳酸菌的产酸能力无显著差异,冻藏第 14、21 和 28 天时,冷冻处理条件下乳酸菌的产酸能力高于冻干处理条件。[结论]冷冻处理更有利于保持酸面团中乳酸菌的活力。

关键词 酸面团;植物乳酸菌;冷冻
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Effect of Frozen and Freeze-drying on the Activity of Lactobacillus in Sourdough

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Abstract [Objective]To study the effect of frozen and freeze-drying on the activity of Lactobacillus in sourdough. [Method]The *Lactobacillus plantarum* M616 was used as the fermentation strain to make sourdough. Then the sourdough were frozen (-80 ℃) and freeze-dried respectively, and were stored at -20 ℃ for 30 days. The survival rate and acid-producing capacity of *L. plantarum* M616, pH and TTA of sourdough were determined. [Result]The results showed that the survival rate of *L. plantarum* M616 in frozen and freeze-dried sourdough was significantly decreased with the prolongation of storage time, but the survival rate of *L. plantarum* M616 in frozen sourdough was significantly higher than that in freeze-dried dough at the same storage time. There was no significantly different between frozen and freeze-dried sourdough on the acid-producing capacity of *L. plantarum* M616 at 0th and 7th day of storage. While on the 14th, 21st and 28th day of storage, the acid-producing ability of *L. plantarum* M616 in frozen sourdough was higher than that of freeze-drying. [Conclusion]Frozen treatment is more conducive to maintaining the activity of *L. plantarum* M616 in sourdough.

Key words Sourdough;*Lactobacillus plantarum*;Frozen

酸面团是由面粉和水混合、在微生物作用下形成的传统面制食品(面包、馒头等)发酵剂。酸面团中微生物种类繁多,乳酸菌是其中典型且重要的一类微生物,乳酸菌在面团发酵过程中产生乳酸等有机酸,赋予食品特有的酸味,同时降低面团 pH,激活面粉中内源蛋白酶,从而产生各类风味物质[1],酸面团发酵会降低本身的黏性和弹性,提高面团的延伸性和松软度,改善食品的质构[2-3]。另外,在酸面团发酵过程中,乳酸菌可以产生多种抗菌物质,抑制面包、馒头中多种霉菌的生长,从而延长食品的货架期[4-5]。最近研究表明,酸面团还可用于制作适合“乳糜泻”人群食用的无面筋食品[6-7]。然而由于乳酸菌代谢的活跃性和酸面团组成成分的复杂性,使酸面团在保存过程中难以实现稳定的品质控制,制约其商品化生产。

目前酸面团在生产中有 3 种常见存在形式:液体、糊状和干燥粉末状,按照发酵工艺的不同又可以分为Ⅰ型、Ⅱ型和Ⅲ型。与Ⅰ、Ⅱ型酸面团不同,Ⅲ型酸面团为干燥的粉状物,多为商业化产品,常用于面包制品的酸味剂、补充剂和香味携带物[4,8]。这种粉末状形式一般通过冷冻干燥或喷雾干燥工艺制备得到,这种方法较好地延长了酸面团的保质期[9]。但是在除去水分的过程中,酸面团中部分风味物质随之减少,乳酸菌的生物活性也会降低[10]。有研究者借鉴冷冻面团技术,将冷冻冻藏工艺应用到酸面团的保存中,这种方法也较好地解决了酸面团不易储藏的问题,但是在冻藏过程中乳酸菌的存活率和产酸能力均有所下降[11]。为了解决上述问题,大量研究集中在抗冻剂或保护剂的筛选和复配方面,并且取得了一定成效。谷胱甘肽、谷氨酸钠、海藻酸钠等一系列添加剂有效地保护了乳酸菌在低温下的生理活性[12-13]。然而,添加剂的使用可能会影响酸面团制品的风味,同时提高了生产成本。

鉴于此,笔者比较了冷冻和冻干 2 种处理方式对酸面团冻藏过程中乳酸菌的存活率及产酸能力的影响,探讨前处理对酸面团冻藏过程中乳酸菌活力的影响,为实现酸面团的稳定品质控制及工业标准化生产提供一定依据。

1 材料与方法

1.1 材料

1.1.1 试验用菌株及面粉。植物乳酸菌 M616,为实验室保藏菌种;面粉,金苑精制粉。

1.1.2 主要仪器和设备。Forma 88000 超低温冰箱,赛默飞世尔科技(中国)有限公司;BTP - 3ESOOX 冷冻干燥机,美国 Virtis 公司;BCD - 227CHT 冰箱,河南新飞电器有限公司;SW - CJ - 1F 单人双面净化工作台,苏州净化设备有限公司;IXFD7 醒发箱,北京东孚久恒仪器技术有限公司;SPX - 150BS - Ⅱ生化培养箱,上海新苗医疗器械制造有限公司;FE20 pH 计,梅特勒 - 托利仪器(上海)有限公司;HQ - 60 - Ⅱ漩涡混合器,北方同正生物科技有限公司;X1 台式离心机,赛默飞世尔科技有限公司。

1.1.3 培养基。MRS 液体培养基:蛋白胨 1.0 g,酵母膏 0.5 g,葡萄糖 2.0 g,三水合乙酸钠 0.5 g,七水合硫酸镁

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0.058 g,牛肉膏 1.0 g,柠檬酸氢二胺 0.2 g,吐温 -80 0.1 mL,磷酸氢二钾 0.2 g,一水合硫酸锰 0.025 g,蒸馏水 100 mL,调节 pH 6.2~6.6[14]。

MRS 固体培养基:MRS 液体培养基的基础上加入琼脂 15 g/L,调节 pH 6.2~6.6。

1.2　方法

1.2.1　乳酸菌的活化及酸面团制备。在无菌条件下吸取植物乳酸菌 M616 于 MRS 液体培养基 37 ℃恒温培养 16 h,然后以 1% 的接种量转接到 100 mL 的 MRS 液体培养基中,37 ℃恒温培养 8 h 后取 35 mL 培养基 3 000 r/min 离心洗涤 2 次备用。将上述乳酸菌与无菌水混合形成 150 g 菌悬液,并与 300 g 面粉均匀混合后切块,分成每个 10 g,放入醒发箱 30 ℃发酵 24 h,制成酸面团。

1.2.2　冷冻酸面团及冻干酸面团的制备。将发酵后的酸面团放入 -80 ℃超低温冰箱冷冻 30 min,取出称量记录,放入 -20 ℃冰箱中备用。将发酵后的酸面团放入 -20 ℃冰箱进行 12 h 的预冻,预冻完成后放入冷冻干燥机进行冷冻干燥,直至样品完全干燥后取出,放入自封袋中称量记录。

1.2.3　乳酸菌存活率的测定。按照国标 GB 4789.2—2010 方法测定活菌落数[15]。

$$\text{乳酸菌存活率} = (\text{冻藏后乳酸菌的活菌数}/\text{冻藏前乳酸菌的活菌数}) \times 100\%$$

1.2.4　pH 及总酸度(TTA)的测定。分别测定 2 种面团第 0、7、14、28 天时的 pH 及 TTA。称取 0.1 g 上述 2 种面团,接入 150 mL MRS 液体培养基,测定培养液 pH。每隔 3 h,分别吸取 2 种乳酸菌菌液 5 mL,用酸度计测定 pH;吸取 10 mL 菌液,加入酚酞,用 0.5 mol/L 氢氧化钠滴定,连续测定 36 h。以测定时间为横坐标、氢氧化钠消耗的体积为纵坐标绘制菌体 TTA 曲线,同时绘制 pH 随时间变化的曲线。

1.2.5　数据分析。该试验中数据均为 3 次平行试验计算得出的平均值,用 SPSS 17.0 及 Origin 8.5.1 进行数据处理及绘图。

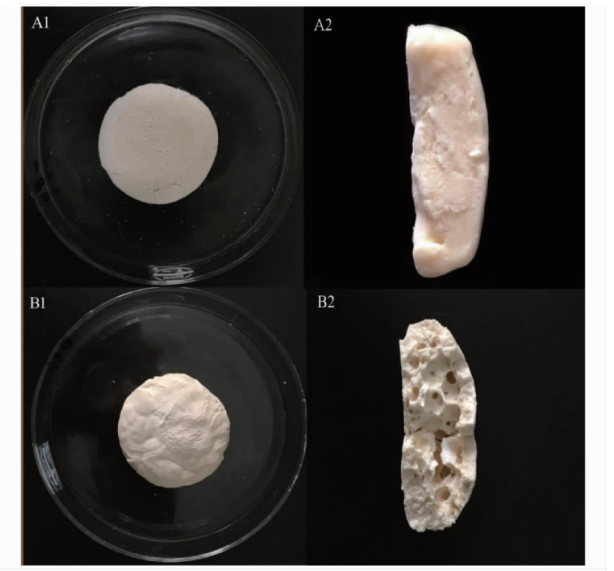
2　结果与分析

2.1　冷冻和冻干处理对酸面团形态的影响　由图 1 可以看出,冻干组酸面团的形态表面皱缩、干燥,从剖视图以看出内部因脱去水分形成许多不规则气孔,质地较脆;冷冻组酸面团的表面光滑有光泽,从剖视图可以看出面团内部光滑有水分,质地较密,黏性较大。

冷冻和冻干的区别在于冻结速度的差异[16],冻干时冻结速度远大于冷冻,从而导致面团内部水分来不及渗出,截留在细胞内,形成大量冰晶,而后的脱水就使得面团形成气孔状结构。

2.2　冷冻和冻干处理对酸面团中乳酸菌存活率的影响　酸面团经过冻干和冷冻处理后乳酸菌活菌数有显著差异。冻藏 7 d 后,稀释度为 10^{-3},冻干和冷冻的活菌落数如图 2 所示。

从图 2 中可以看出,冷冻组活菌落数显著高于冻干组,冻干处理的酸面团中乳酸菌的活菌落数为 68 个,而冷冻组

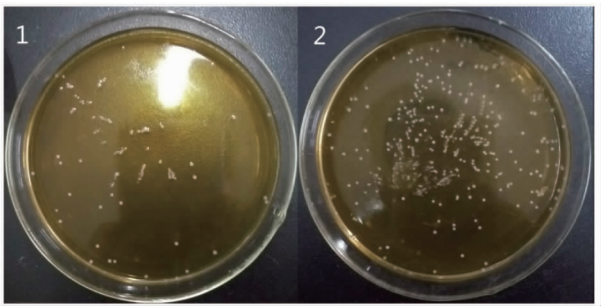


注:A. 外部;B. 内部;1. 冷冻;2. 冻干

Note:A. exterior;B. interior;1. frozen;2. Freeze-drying

图 1　冷冻、冻干酸面团表面和内部结构

Fig. 1　Surface and internal structure of frozen and freeze - drying sourdough



注:1. 冻干;2. 冷冻

Note:1. freeze-drying;2. frozen

图 2　酸面团中乳酸菌活菌落数

Fig. 2　Viable colonies of lactic acid in sour dough

的为 182 个。这说明冻干会使酸面团中大量乳酸菌失活。2 种处理方式对酸面团中乳酸菌存活率的影响如图 3 所示。酸面团经过冻干处理,冷藏 7、14、21、28 d 后测得的乳酸菌的存活率分别为 12.3%、10.7%、8.6%、7.0%,而经过冷冻处理的酸面团冷藏 7、14、21、28 d 后测得的乳酸菌的存活率分别为 42.3%、20.0%、15.3%、12.7%。可以看出,2 种处理方式乳酸菌存活率均逐渐下降,随着冻藏时间的延长,两者差异逐渐缩小,但冷冻组乳酸菌存活率均显著高于冻干组。

冻干处理时冻结速度较快,由此产生的机械损伤和溶质效应会损伤细胞膜,造成乳酸菌的死亡[17],同时冷冻处理也会由于溶质效应损伤乳酸菌。从图 3 可以看出,冻干过程中已经造成了大量的乳酸菌失活,而冷冻处理乳酸菌失活较少,在冻藏过程中由于复水及冻融影响,乳酸菌逐渐失活[18-19]。这可以从存活率的变化上清晰看出,冻干组存活率降低幅度较小,而冷冻组第 14 天的存活率较第 7 天下降了 52.7%,而冷冻组仅下降了 11.4%。

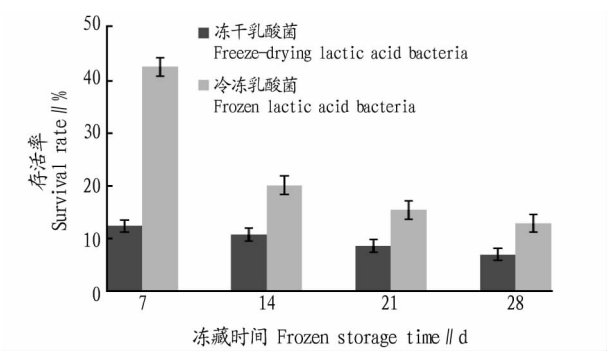
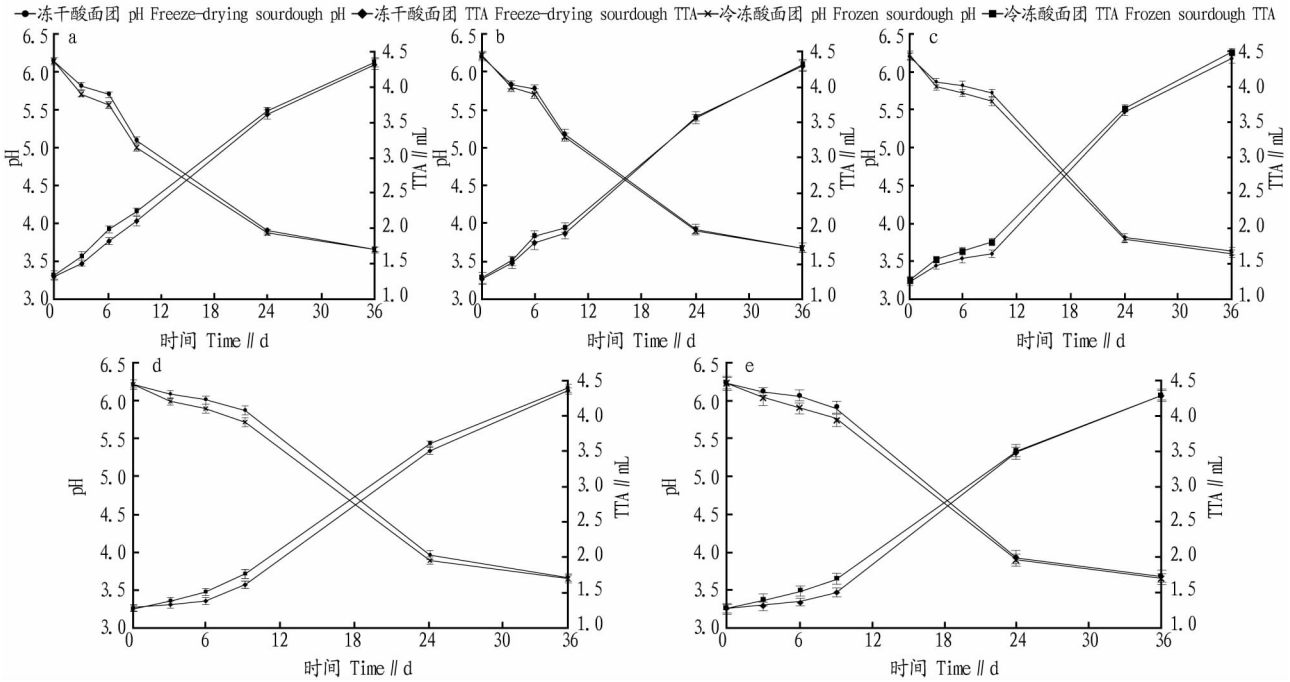


图 3 冷冻和冻干处理方式对酸面团中乳酸菌存活率的影响

Fig. 3 Effect of frozen and freeze-drying on lactic acid bacteria survival in sourdough

2.3 冷冻和冻干处理对酸面团中乳酸菌产酸能力的影响 选取 -20 ℃冻藏第 0、7、14、21 及 28 天的 2 组样品面团进行 pH 及 TTA 的测定,结果如图 4 所示。由图 4 可以看出,随着时间的延长,面团 pH 逐渐降低,TTA 逐渐升高。第 0 天及第 7 天时的 pH 及 TTA 前 9 h 变化幅度较第 14、21 及 28 天大,说明第 0 天及第 7 天面团中乳酸菌活性较高,产酸能力强,第 14、21 及 28 天时面团 pH 及 TTA 变化趋势基本一致,说明此时的乳酸菌产酸能力差异不大,这个结果印证了图 3 中数据。随着冻藏时间的延长,pH 及 TTA 数值在 0、24、36 h 时差异不显著,冷冻组 pH 及 TTA 数值在 3 ~ 9 h 时均存在显著差异,说明乳酸菌产酸阶段集中在 3 ~ 9 h[11],此时冷冻组面团中乳酸菌产酸能力强于冻干组。



注:a. 第 0 天;b. 第 7 天;c. 第 14 天;d. 第 21 天;e. 第 28 天。

Note: a. day 0; b. day 7; c. day 14; d. day 21; e. day 28

图 4 冻藏过程中酸面团 pH 及 TTA 变化

Fig. 4 Changes of pH and TTA in sourdough during frozen storage

3 结论

该研究通过比较 -80 ℃冷冻及冻干 2 种处理方式对酸面团外观形态及冻藏期内其中乳酸菌存活率及产酸能力,认为冷冻处理更有利于酸面团实现稳定品质控制。冷冻处理不仅可以维持酸面团良好的形态,更有利于保持乳酸菌的活力,获得良好的品质。

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