

## Original Research Article

# Production of lactic and acetic acids during fermentation of milk fortified with kiwi juice using *Saccharomyces boulardii* and lactobacilli

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### Abstract

**Purpose:** To investigate the synergistic effect of *Saccharomyces boulardii* and lactobacilli on lactic and acetic acids produced during fermentation of milk fortified with kiwi juice, relative to fermentation of unfortified milk.

**Methods:** Skimmed milk was fortified with kiwi juice (4 % v/v) and fermented for 12 h at 37 °C by a combination of *S. boulardii* and lactobacilli strains. Lactic and acetic acids were determined using gas chromatography-mass spectrometry (GS-MS).

**Results:** The presence of kiwi juice in the milk stimulated the production of lactic (1.35 g/100g) and acetic (0.29 mg/g) by *S. boulardii* in the absence of lactobacilli. When *S. boulardii* was inoculated with *Lb. casei* 20975, the production of lactic acid and acetic acid increased to 2.36 g/100 g and 0.71 mg/g, respectively. Furthermore, acid production increased when *Lb. plantarum* RS (35-11), *Lb. casei* LCS, and *Lb. plantarum* JXJ (6 - 12) were inoculated into milk free of kiwi juice in which *S. boulardii* was grown. *Saccharomyces boulardii* resulted in marginal production of acids by *Lb. fermentum* F9.

**Conclusion:** These results show that acid production is positively affected by some lactobacilli strains in the milk whether fortified with kiwi juice or free of this juice. However, fermentation of these formulations for a period longer than 6 h may result in losses in acid yield.

**Keywords:** Fermented milk, Kiwi juice, *Saccharomyces boulardii*, Lactobacilli, Lactic acids, Acetic acid

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## INTRODUCTION

*Saccharomyces boulardii* was first isolated from lychees fruit (*Litchi chinensis*) by a French microbiologist, Henri Boulard in Indochina where lychees grow and prefer warm tropical climate [1]. *Saccharomyces boulardii* is used in many

fermented dairy products e.g. acidophilus milk [2]. Fermented dairy products are good vehicles for *Lactobacillus* strains since these cultures remain metabolically efficient [3]. *Lactobacillus* constitutes the largest genus of LAB: over 125 species and sub-species have been identified and recognized. A major characteristic of LAB is

the capacity to generate lactic acid and acetic acid as the principal or only fermentation products [4].

*Lactobacillus* species (e.g. *casei*, *plantarum* and *fermentum*) are considered ubiquitous bacteria that can be used largely as culture or in mixtures in traditional dairy products. Therefore, the production of fermented dairy products inoculated with *Lactobacillus* strains has been developed in the last decade [3,5,6]. Fortification of milk with fruit juices (e.g., kiwi) has been reported to increase the bioavailability of nutrients [7]. Furthermore, *lactobacilli* are able to ferment fruit juices such as kiwi spontaneously, which reveals their reliability and consistency of performance.

Although yeasts have been considered as an integral part of the microflora of many dairy-related products, the active use of yeasts as dietary adjuncts for *lactobacilli* has been limited. However, *Kefir* is an exception since it is a fermented milk-based beverage manufactured using yeasts and bacteria [5]. In addition, *S. boulardii* could grow to a great extent in combination with the starter culture bacteria after inoculation in a dairy product such as yogurt [8]. Therefore, the synergistic effects of *S. boulardii* and *lactobacilli* on dairy products have been reported [9]. It has been also reported that one of these synergistic effects is the optimal growth temperature of 37 °C since it is about the same whether in the presence of *S. boulardii* alone or in combination with *lactobacilli* [10].

The combination of *S. boulardii* with *lactobacilli* by inoculation of the yeast into commercial milk products, has been demonstrated to enhance the metabolic activities of the microorganisms [6]. Although some studies have reported that *S. boulardii* may not be capable of lactose fermentation [11], it has been shown that the metabolism of *lactobacilli* during fermentation of dairy products is stimulated by the fermentative functions of *S. boulardii* [6,8].

The organic acids produced by *lactobacilli* are known to affect *Saccharomyces* metabolism if they are inoculated together [12]. Nevertheless, the organic acids are not always produced by *lactobacilli* alone because the yeast also generates the same acids as minor products of metabolism [10].

The main purpose of this study was to determine the rates of lactic and acetic acids production by *S. boulardii* alone or in combination with *lactobacilli* during fermentation of milk fortified with kiwi juice or free of this juice.

## EXPERIMENTAL

### Materials

Skimmed milk powder was purchased from Guang Ming Co. Ltd (Wenzhou, China). Kiwi (*Actinidia chinensis*) was purchased from a local retail market in China, while MF-Millipore membrane filter (mixed cellulose esters, asbestos monitoring, 0.22 µm, 25 mm, white, gridded) was purchased from Sigma-Aldrich (Shanghai, China). All chemicals used in the experiments were of analytical grade and of high purity.

### Microorganisms and media

*Saccharomyces boulardii* (Reflor; Biocodex, Cedex, France), and all of the *Lactobacillus* strains used in this study were obtained from the Synergistic Innovation Center for Food Safety and Nutrition, Jiangnan Uni. (Wuxi, China). Yeast extract peptone dextrose (YPD) was used to activate *S. boulardii*, while DeMan, Rogosa, Sharpe (MRS) was used to activate *lactobacilli*.

### Preparation of the media substrates and fermentation

Skimmed milk powder was reconstituted at a concentration of 11 % (w/v), pasteurized for 15 sec at 72 °C, and then sterilized using MF-Millipore membrane filter (0.22 µm/25 mm). Kiwi Juice was extracted using Santos blender and sterilized using MF-Millipore membrane filter (0.22 µm/25 mm). The milk was fortified with kiwi juice to a level of 4 % (v/v). The formulations of skimmed milk fortified with kiwi juice, and skimmed milk free of this juice were distributed in 150 mL quantities into sterile Erlenmeyer flasks (250 mL). They were then inoculated with an active culture of *S. boulardii*, alone or together with *S. boulardii* and active culture of *lactobacilli* strains. The inoculated substrates were allowed to ferment for 12 h at 37 °C under anaerobic conditions. Samples were withdrawn at regular intervals for analysis.

### Gas chromatography-mass spectrometry

Derivatization of analyte for GC-MS was performed according to a previous protocol [13] with few modifications. Lactic and acetic acids were determined with Thermo/GC-MS/Trace 1310 (TSQ 8000 Evo, Switzerland) using a column of the Rtx-5MS capillary (dimensions: 30 m, 0.25 mm /0.25 µm). The carrier gas was helium at a flow rate of 35.0 cm<sup>3</sup>/sec. The initial temperature was 320 °C (90 °C/min), and was held for 5 min.

## Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD) of seven treatments (triplicate samples) using SPSS 19.0 software. Significant differences between treatment groups were determined using Duncan's multiple ranges. Values of  $p < 0.05$  were assumed significant.

## RESULTS

The natural amounts of lactic acid and acetic acid detected in all of the treatments prior to fermentation were 0.098 – 0.102 g/100g and 0.085 – 0.086 mg/g, respectively (Tables 1 & 2). For milk fortified with kiwi juice, *S. boulardii* and the facultative hetero-fermentative *Lb. casei* 20975 together significantly increased yields of lactic acid and acetic acid to maximum values of 2.36 g/100g and 0.71 mg/g, respectively. When kiwi juice-free milk was inoculated with *S. boulardii* and 20975 together, the production of acids still occurred and was higher when compared with other treatments, but was reduced to 2.02 g/100g for lactic, and 0.63 mg/g for acetic acid. Remarkably, the amount of acids produced increased in milk free of added kiwi juice, when compared with kiwi-fortified milk where *S. boulardii* was inoculated with the facultative hetero-fermentative *Lb. casei* LCS, *Lb. plantarum* RS (35 - 11) or even *Lb. plantarum* JXJ (6 - 12).

These facultative hetero-fermentative strains increased lactic acid production within 6 h (1.754.81 g/100g) and differed significantly ( $p < 0.05$ ), when compared with the other treatments. The amount of acetic acid produced increased to  $\leq 0.56$  mg/g with *S. boulardii* when inoculated with RS (35-11) or JXJ (6-12), but was greater than 0.23 mg/g observed for LCS. On the other hand, the presence of kiwi juice in milk resulted in increased acid production (1.35 g/100g for lactic, and 0.29 mg/g for acetic) when *S. boulardii* was inoculated alone. However, the increase was not significant ( $p > 0.05$ ), when compared with the case where *Lb. plantarum* RS (35-11) was present. In milk with or without added kiwi juice, acid production was as low as  $\leq 1.21$  g/100g (for lactic) and  $\leq 0.16$  mg/g (for acetic acid) when *S. boulardii* was cultured together with the obligate hetero-fermentative *Lb. fermentum* F9. The obligate hetero-fermentative *Lb. fermentum* F16 was more compatible with *S. boulardii* than with F9 when it was inoculated together into kiwi-based milk, resulting in increased production of acids to a maximum of 1.71 g/100g (for lactic acid), and 0.26 mg/g (for acetic acid). However, these did not differ

significantly, when compared with RS (35 – 11) ( $p > 0.05$ ).

## DISCUSSION

Lactic and acetic acids are mainly produced by microorganisms through breakdown of milk lactose [14]. In milk fortified with kiwi juice or milk free of this juice, inoculation with *S. boulardii* and *lactobacilli* increased the acid production rapidly and to a higher degree, relative to when *S. boulardii* was used alone. However, the acid production declined early after reaching maximum levels within 6 h. This was to be expected since the competition from the *S. boulardii* caused a stress on *lactobacilli* metabolism.

Moreover, acid production by *S. boulardii* in milk free of added kiwi juice (control) seemed to be triggered by the unavailability of fermentable sugars, and it may be indicative of the doubtful capability of *S. boulardii* to ferment lactose and produce acids [15]. This was to be expected since *S. boulardii* produced acids marginally when it was allowed to grow in the absence of *lactobacilli*. However, the production of acids by *S. boulardii* alone increased when the milk was fortified with kiwi juice, indicating availability of fermentable sugars for yeast metabolism. Therefore, the increased acid production was associated with presence of *S. boulardii* alone, indicating the acidic environment caused by the presence of kiwi juice.

However, it must be pointed out that the relatively increased production of acids in the presence of the *lactobacilli* might have been employed by *S. boulardii* as a carbon source. After reaching maximum levels, the production of acids declined as rapidly as when *lactobacilli* was present, indicating predictable uptake of the acids by *S. boulardii*. Therefore, *lactobacilli* could be much more compatible with *S. boulardii* in milk free of kiwi juice, or even kiwi-based milk.

As expected, the acidity of kiwi juice induced microbial consumption of the fermentable sugars, and resulted in increased acid production during fermentation by yeast and *lactobacilli*. On the other hand, it has been suggested that the high acidity of yeast vehicle might pose a potent inhibition to the viability of *Saccharomyces* [16]. However, this is not consistent with the results of the present study. The enhanced production of acids by *Lb. casei* as reported here may be related to the vitamins contained in the kiwi juice.

**Table 1:** Changes in lactic acid content (g/100g) during fermentation at 37 °C for 12 h by *Saccharomyces boulardii*, or by a combination of *S. boulardii* and *Lactobacilli* strains

Treatments*							
Media substrate: milk alone (Control)							
Period (h)	<i>S. boulardii</i>	<i>S. boulardii</i> +20975	<i>S. boulardii</i> +LCS	<i>S. boulardii</i> +RS	<i>S. boulardii</i> +JXJ	<i>S. boulardii</i> +F9	<i>S. boulardii</i> +F16
0	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>
2	0.12 ± 0.02 <sup>e</sup>	1.76 ± 0.02 <sup>a</sup>	1.71 ± 0.08 <sup>a</sup>	1.76 ± 0.04 <sup>a</sup>	1.34 ± 0.02 <sup>c</sup>	0.76 ± 0.01 <sup>d</sup>	1.49 ± 0.03 <sup>b</sup>
4	0.13 ± 0.01 <sup>e</sup>	1.94 ± 0.04 <sup>a</sup>	1.74 ± 0.09 <sup>b</sup>	1.78 ± 0.02 <sup>b</sup>	1.75 ± 0.01 <sup>b</sup>	1.10 ± 0.03 <sup>d</sup>	1.57 ± 0.05 <sup>c</sup>
6	0.14 ± 0.01 <sup>e</sup>	2.02 ± 0.01 <sup>a</sup>	1.75 ± 0.04 <sup>b</sup>	1.81 ± 0.04 <sup>b</sup>	1.79 ± 0.03 <sup>b</sup>	1.12 ± 0.03 <sup>d</sup>	1.68 ± 0.06 <sup>c</sup>
8	0.15 ± 0.01 <sup>e</sup>	1.88 ± 0.04 <sup>a</sup>	1.62 ± 0.02 <sup>c</sup>	1.78 ± 0.01 <sup>b</sup>	1.76 ± 0.01 <sup>b</sup>	1.11 ± 0.04 <sup>d</sup>	1.65 ± 0.03 <sup>c</sup>
10	0.15 ± 0.02 <sup>g</sup>	1.81 ± 0.02 <sup>a</sup>	0.94 ± 0.03 <sup>e</sup>	1.22 ± 0.02 <sup>d</sup>	1.62 ± 0.05 <sup>b</sup>	0.32 ± 0.05 <sup>f</sup>	1.30 ± 0.06 <sup>c</sup>
12	0.12 ± 0.01 <sup>f</sup>	0.94 ± 0.03 <sup>b</sup>	0.22 ± 0.04 <sup>e</sup>	0.34 ± 0.04 <sup>d</sup>	1.12 ± 0.02 <sup>a</sup>	0.18 ± 0.01 <sup>e</sup>	0.81 ± 0.02 <sup>c</sup>
Media substrate: kiwi juice-fortified milk (4% v/v)							
Period (h)	<i>S. boulardii</i>	<i>S. boulardii</i> +20975	<i>S. boulardii</i> +LCS	<i>S. boulardii</i> +RS	<i>S. boulardii</i> +JXJ	<i>S. boulardii</i> +F9	<i>S. boulardii</i> +F16
0	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>
2	0.52 ± 0.04 <sup>d</sup>	1.52 ± 0.02 <sup>a</sup>	1.20 ± 0.01 <sup>c</sup>	1.28 ± 0.04 <sup>b</sup>	1.32 ± 0.05 <sup>b</sup>	0.34 ± 0.03 <sup>e</sup>	1.28 ± 0.01 <sup>b</sup>
4	0.62 ± 0.05 <sup>f</sup>	2.02 ± 0.04 <sup>a</sup>	1.54 ± 0.03 <sup>d</sup>	1.69 ± 0.02 <sup>c</sup>	1.75 ± 0.02 <sup>b</sup>	0.95 ± 0.04 <sup>e</sup>	1.59 ± 0.01 <sup>d</sup>
6	1.09 ± 0.02 <sup>e</sup>	2.36 ± 0.04 <sup>a</sup>	1.61 ± 0.01 <sup>c</sup>	1.73 ± 0.02 <sup>b</sup>	1.69 ± 0.06 <sup>b</sup>	1.21 ± 0.02 <sup>d</sup>	1.71 ± 0.05 <sup>b</sup>
8	1.33 ± 0.02 <sup>d</sup>	2.21 ± 0.02 <sup>a</sup>	1.36 ± 0.04 <sup>d</sup>	1.65 ± 0.05 <sup>b</sup>	1.53 ± 0.01 <sup>c</sup>	0.71 ± 0.05 <sup>e</sup>	1.63 ± 0.02 <sup>b</sup>
10	1.35 ± 0.04 <sup>b</sup>	1.92 ± 0.05 <sup>a</sup>	1.11 ± 0.02 <sup>d</sup>	1.34 ± 0.04 <sup>b</sup>	1.13 ± 0.03 <sup>d</sup>	0.27 ± 0.02 <sup>e</sup>	1.27 ± 0.01 <sup>c</sup>
12	0.46 ± 0.03 <sup>c</sup>	1.64 ± 0.05 <sup>a</sup>	0.22 ± 0.01 <sup>d</sup>	0.46 ± 0.02 <sup>c</sup>	0.40 ± 0.04 <sup>c</sup>	0.13 ± 0.01 <sup>e</sup>	0.61 ± 0.06 <sup>b</sup>

\*Data are expressed as mean ± standard deviation (SD) of seven treatments (triplicate samples) which were analyzed in each treatment. Different superscript letters (<sup>a-g</sup>) in the same row denote significant differences ( $p < 0.05$ ) among treatments for the same fermentation period

**Table 2:** Changes in acetic acid content (mg/g) during fermentation at 37 °C for 12 h by *Saccharomyces boulardii*, or by a combination of *S. boulardii* and *lactobacilli* strains

Period (h)	Treatments*						
	Media substrate: milk alone (Control)						
	S. <i>boulardii</i>	S. <i>boulardii</i> +20975	S. <i>boulardii</i> +LCS	S. <i>boulardii</i> +RS	S. <i>boulardii</i> +JXJ	S. <i>boulardii</i> +F9	S. <i>boulardii</i> +F16
0	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>
2	0.09 ± 0.00 <sup>d</sup>	0.54 ± 0.01 <sup>a</sup>	0.14 ± 0.03 <sup>d</sup>	0.36 ± 0.05 <sup>c</sup>	0.42 ± 0.03 <sup>b</sup>	0.10 ± 0.02 <sup>d</sup>	0.12 ± 0.04 <sup>d</sup>
4	0.09 ± 0.01 <sup>d</sup>	0.61 ± 0.02 <sup>a</sup>	0.14 ± 0.02 <sup>cd</sup>	0.45 ± 0.05 <sup>b</sup>	0.48 ± 0.05 <sup>b</sup>	0.10 ± 0.03 <sup>d</sup>	0.18 ± 0.02 <sup>c</sup>
6	0.09 ± 0.01 <sup>d</sup>	0.63 ± 0.04 <sup>a</sup>	0.23 ± 0.01 <sup>c</sup>	0.54 ± 0.01 <sup>b</sup>	0.56 ± 0.04 <sup>b</sup>	0.10 ± 0.04 <sup>d</sup>	0.18 ± 0.02 <sup>c</sup>
8	0.09 ± 0.00 <sup>e</sup>	0.61 ± 0.01 <sup>a</sup>	0.22 ± 0.02 <sup>d</sup>	0.42 ± 0.03 <sup>c</sup>	0.55 ± 0.04 <sup>b</sup>	0.09 ± 0.02 <sup>e</sup>	0.18 ± 0.04 <sup>d</sup>
10	0.04 ± 0.00 <sup>d</sup>	0.47 ± 0.02 <sup>a</sup>	0.22 ± 0.01 <sup>c</sup>	0.32 ± 0.01 <sup>b</sup>	0.47 ± 0.03 <sup>a</sup>	0.08 ± 0.03 <sup>d</sup>	0.05 ± 0.01 <sup>d</sup>
12	0.04 ± 0.00 <sup>d</sup>	0.36 ± 0.04 <sup>a</sup>	0.08 ± 0.02 <sup>c</sup>	0.11 ± 0.01 <sup>c</sup>	0.26 ± 0.01 <sup>b</sup>	0.03 ± 0.01 <sup>d</sup>	0.04 ± 0.03 <sup>d</sup>
Media substrate: kiwi juice-fortified milk (4% v/v)							
	S. <i>boulardii</i>	S. <i>boulardii</i> +20975	S. <i>boulardii</i> +LCS	S. <i>boulardii</i> +RS	S. <i>boulardii</i> +JXJ	S. <i>boulardii</i> +F9	S. <i>boulardii</i> +F16
0	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>
2	0.12 ± 0.01 <sup>d</sup>	0.52 ± 0.01 <sup>a</sup>	0.09 ± 0.01 <sup>d</sup>	0.11 ± 0.04 <sup>d</sup>	0.17 ± 0.03 <sup>b</sup>	0.13 ± 0.02 <sup>cd</sup>	0.16 ± 0.02 <sup>bc</sup>
4	0.12 ± 0.00 <sup>d</sup>	0.64 ± 0.04 <sup>a</sup>	0.15 ± 0.01 <sup>d</sup>	0.17 ± 0.03 <sup>d</sup>	0.31 ± 0.01 <sup>b</sup>	0.15 ± 0.03 <sup>d</sup>	0.25 ± 0.04 <sup>c</sup>
6	0.13 ± 0.00 <sup>d</sup>	0.71 ± 0.03 <sup>a</sup>	0.16 ± 0.02 <sup>d</sup>	0.22 ± 0.03 <sup>bc</sup>	0.18 ± 0.04 <sup>cd</sup>	0.16 ± 0.02 <sup>d</sup>	0.26 ± 0.04 <sup>b</sup>
8	0.26 ± 0.01 <sup>b</sup>	0.66 ± 0.03 <sup>a</sup>	0.13 ± 0.01 <sup>c</sup>	0.14 ± 0.02 <sup>c</sup>	0.13 ± 0.02 <sup>c</sup>	0.13 ± 0.05 <sup>c</sup>	0.22 ± 0.03 <sup>b</sup>
10	0.29 ± 0.00 <sup>b</sup>	0.54 ± 0.04 <sup>a</sup>	0.06 ± 0.02 <sup>d</sup>	0.08 ± 0.01 <sup>d</sup>	0.07 ± 0.02 <sup>d</sup>	0.06 ± 0.01 <sup>d</sup>	0.16 ± 0.02 <sup>c</sup>
12	0.07 ± 0.00 <sup>b</sup>	0.25 ± 0.03 <sup>a</sup>	0.05 ± 0.01 <sup>bc</sup>	0.06 ± 0.02 <sup>bc</sup>	0.05 ± 0.01 <sup>bc</sup>	0.03 ± 0.02 <sup>c</sup>	0.07 ± 0.01 <sup>b</sup>

Data are expressed as mean ± standard deviation (SD) of seven treatments (triplicate samples) which were analyzed in each treatment. Different superscript letters (<sup>a-g</sup>) in the same row denote significant differences ( $p < 0.05$ ) among treatments for the same fermentation period

An association has been suggested between high production of acids and the optimum fermentation temperature of 37 °C for the metabolic activity of *Lb. casei* and *Lb. fermentum* [17, 18]. Kiwi minerals (e.g. magnesium) might also act as a prebiotic constituent, thus inducing acid production by *lactobacilli*.

In milk free of added kiwi juice, it was not clear whether the high production of acids was due to *S. boulardii* or metabolism of *Lb. plantarum* [RS (35-11), JXJ (6-12)] and *Lb. casei* LCS, or both. It is known that *Lb. plantarum* and *Lb. casei* are facultative hetero-fermentative strains with capability to produce acids [19]. However, this does not rule out the possibility of acid production by *S. boulardii* [10], particularly since its level of acid production was substantial in the presence of kiwi juice. For this reason, few curds were aggregated in milk fortified with kiwi juice where *Lb. plantarum* [RS (35-11), JXJ (6-12)] and *Lb. casei* 20975 were present. The

aggregation has been attributed to increased acid production caused by alterations in the electrostatic interactions in the protein network [20]. The solubility seen in the other treatments might due to low acid production.

## CONCLUSION

This study addressed the stimulatory effect of fortification of milk with kiwi juice on acid production by yeast and some strains of *lactobacilli*. Although acid production was positively influenced by some of *lactobacilli* strains in the milk (whether fortified with kiwi juice or free of this juice), fermentation of these formulations for periods longer than 6 h may result in losses in acid yield. It is not obvious whether the negligible production of acetic acid was due to the slow speed of metabolism in some of the *lactobacilli*, or whether it was as a result of increased consumption of the acids by

*S. boulardii*. Further investigations are required to ascertain the true situation.

## DECLARATIONS

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### Conflict of interest

No conflict of interest is associated with this work

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hao Zhang conceived and designed the study. Gang Wang designed all the experiments and revised the manuscript. Ahmed Hassan Mousa performed the experiments, collected, analyzed the data, and wrote the manuscript.

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