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Promotional effects on naturally occurring lactic acid bacteria without impairing chickpea germination

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Abstract: Sprouting has been used widely to enrich the nutritional quality of cereals and legumes. It improves the bioavailability of nutrients, especially those bound to phytic acid. However, sprouting is a good medium for microbial growth; thus, producing safe sprouts from harmful microbial growth is challenging. In food biotechnology, lactic acid bacteria (LAB) can be potentially used to improve nutrition and play a vital role as competitive microbes in food preservation. Therefore, supporting natural LAB growth by adding glucose sources during sprouting can produce a safer sprouting medium. Chickpeas (*Cicer arietinum* L.) sprouted for up to 50 h with glucose (0.1% and 1%) under aero-anaerobic conditions, with recycled water periodically spraying on the sprouts to support the natural LAB growth. Results show increased LAB counts, lactic acid and acetic acid, and decreased pH. Moreover, the addition of glucose had no significant detrimental effects on sprout quality compared to the control sample relative to nutritional compounds, such as saccharides, which remained similar. This sprouting method can be scaled up to production levels and is considerably cheaper than other treatments.

Keywords: biological treatment; *Cicer arietinum* L.; organic acids; phytic acid; sprouts

Healthy living trends and healthy food themes have pushed the food industry to seek new natural and safe farm-to-fork production methods, e.g. nutraceuticals and functional foods such as microgreens, gluten-free diets, and organic products (Turner et al. 2020). Germination is one of the most effective and cheap ways to improve the nutrient quality of legumes. It increases nutrients and decreases anti-nutritional factors in sprouts (Nkhata et al. 2018). Chickpea (*Cicer arietinum* L.) is one of the most common pulses in the human diet (Wood and Grusak 2007). It also increases nutritional and bioactive compounds such as crude protein and essential amino acids, crude fat, and ascorbic acid (Camacho et al. 1992; Khalil et al. 2007; Ferreira et al. 2019); phenolic compounds (Xu et al. 2018); cellulose and hemicellulose (Vasishtha and Srivastava 2013); and minerals such as sodium (Na), magnesium (Mg), iron (Fe), and zinc (Zn) (Atudorei et al. 2021). Additionally, chickpea germination decreases anti-nutrients such as galactooligosaccharides, phytate, tannin, and trypsin inhibitors (Neves and Lourenco 2001; Tewari 2002; Haileslassie et al. 2019).

Despite the numerous advantages of chickpea germination, ensuring safe germination relative to unwanted microbiological growth can be problematic. Many biological, chemical, and physical treatments

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have been developed to solve this problem, especially treating seeds before germination (Ding et al. 2013; Sikin et al. 2013; Yang et al. 2013). However, no one treatment perfectly solves the problem without affecting germination rates, reducing cost-effectiveness, introducing adverse health effects, or not being widely accepted by consumers (Yang et al. 2013; Benincasa et al. 2019). The most critical stage of the germination process is soaking and germination since these create favourable conditions (high humidity, room temperature, low light, nutrient abundance) for the growth and persistence of pathogens (NACMCF 1999; Turner et al. 2020). Therefore, treatment during this time could be beneficial for preventing undesirable microbial growth, for instance, by controlling the acidity of the sprouting water. Chemical and physical treatments are more commonly used in this stage than physiological treatments. However, added chemicals create the risk of residues or deterioration of sprout yield and quality while still not reducing microbial growth to the desired levels. For instance, using acidic electrolysed water or organic acids during germination fails to produce a 3-log reduction (Nei et al. 2014; Zhang et al. 2019).

This work explores the supporting effect of glucose addition to the natural LAB on chickpea sprouting in aero-anaerobic conditions with recycled water for up to 50 hours. Our findings are expected to provide survival and increased LAB count and its product, such as lactic acid, without significantly reducing sprout yield.

MATERIAL AND METHODS

Sprouting. Chickpea (*Cicer arietinum* L.) type Kabuli seeds (light yellow coated) were purchased from a local market.

Seeds were soaked in tap water (1:6 *w*/*v*) for 12 h at ambient temperature (22–23 °C). After soaking, seeds were divided for simultaneous sprouting into three batches (i.e. sprouters); Freshlife FL-3000 Automatic Sprouters (Tribest Corporation, USA) were used for sprouting. One of the three batches was the control sample, which used plain tap water for germination (control) as the rinse water. The other two sprouters used rinse water with different concentrations of glucose (i.e. 0.1% – Glu 1 and 1% – Glu 2, *w*/*w*) (Fluka, Germany). The sprouting systems of all groups were the same, and the rinse water was not changed during the sprouting period (50 h). The sprouting system contains an aerobic (above the reservoir water line) part and an oxygen limiting (below the reservoir water line) part (where natural microbial growth, especially LAB, was supported) (Figure 1). Sprouting was done at room temperature.

Physiological measurement. Sprout length was measured at every time point of sample collection (i.e. at 0, 26, and 50 h) using a calliper (Somet, Czechoslovakia). Twenty seeds, five from each of four distinct locations, were measured within the germinator. Acidity was measured using a pH meter (Testo 206; Testo, Germany). Measurements were done according to the Official Methods of Analysis AOAC 925.09 (2000). Moisture analysis was measured and determined according to the AOAC 925.09 (2000).

Microbiological analysis. The representative samples (about 20 g from each sprouting device, see Figure 1) were taken aseptically at 0, 26, and 50 h of germination. De Man, Rogosa, and Sharpe (MRS) agar (Oxoid, UK) were used for the cultivation of lactic acid bacteria (LAB). Results are presented as the number of colony forming units (CFU) per 1 g of sample.

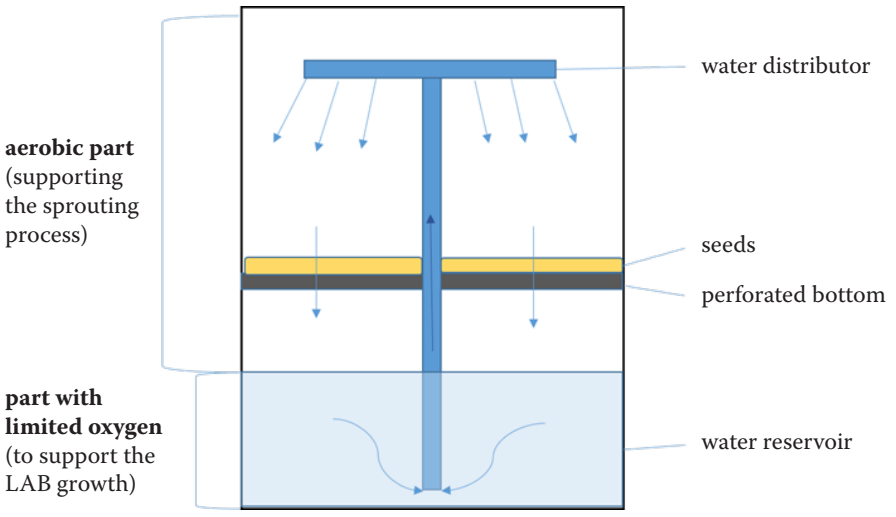

Figure 1. The schematic of the sprouting system

LAB – lactic acid bacteria

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Chemical analysis. Phytic acid content was determined using the K-PHYT 05/19 kit (Megazyme, Ireland). Phytic acid content was converted to the amount in dry matter. Germination water and chickpea samples were analysed for organic acids by HPLC-C18-DAD, Agilent Infinity II with Agilent Poroshell 120 EC C18 (Agilent, USA), and carbohydrates by HPLC-HILIC-RID, Agilent Infinity II with Agilent Zorbax NH2 (Agilent, USA). Results were reported as % *w*/*w*.

Statistical analysis. Data were analysed using SPSS software (version 28.0) at a significance level of 5%. Normality was tested using Shapiro-Wilks and histograms; Q-Q plots were used to confirm the normality of the variables. Multivariate General Linear Models (GLMs) were used to test the effects of the glucose treatment. The non-parametric Kruskal Wallis test was used to analyse the non-normally distributed parameters.

RESULTS AND DISCUSSION

The sprouting system combined aerobic conditions (upper part) and oxygen-limiting conditions (rinse water reservoir) (Figure 1). The primary goal of this arrangement was to support natural LAB on the seed surface by adding glucose to the rinse water irrigation system. LAB has multiple roles as a biological treatment, e.g. a competitive microbe against other undesirable microbes or organic acid production such as lactic acid and acetic acid (Šušković et al. 2010; Ding et al. 2013; Sikin et al. 2013). Moreover, adding glucose supports the germination process by providing an exogenous source of carbohydrates for energy during sprouting (Guglielminetti et al. 1995). Thus, germination and fermentation could have occurred during the sprouting period. Both processes involve changes in sprouting media and metabolites in the chickpea seeds (Nkhata et al. 2018). The only study on adding sugar during sprouting focused mainly on adding sucrose to improve the production of phenolic compounds. For instance, sucrose treatment of mung bean seeds was studied relative to increasing the levels of vitamin C, total phenolic content, and antioxidant activity (Wei et al. 2019). This study used a sprouting system without an oxygen-limiting reservoir, and the paper did not discuss the effect of the microbial population on the seeds.

Effects of sprouting conditions on germination physiology, LAB, and sprout growth. The statistic GLMs showed the significant effects of the glucose treatment on decreasing the pH of the rinse water ($P < 0.001$) and the seeds ($P < 0.001$) and increasing the LAB count on the seeds ($P = 0.037$) (Table 1). The culti-

Table 1. General Linear Models (GLMs) show significant differences between glucose treatments, sprouting times, and treatment × sprouting time in pH, moisture, microbial counts, sprout length, and phytic acid

Parameters	Glucose treatments			*P*-value	Sprouting time (h)			*P*-value	Treatment × sprouting time	SEM
	control	Glu 1	Glu 2		0	26	50		*P*-value	
pH rinse water	6.91^{a}	5.17^{b}	3.71^{c}	< 0.001	7.39^{a}	5.17^{b}	5.19^{b}	0.044	< 0.001	0.148
pH seed	6.42^{a}	6.08^{a}	5.52^{b}	< 0.001	6.58^{a}	6.00^{ab}	5.50^{b}	< 0.001	0.024	0.144
Moisture (%)	59.77	59.55	59.34	0.8090	57.87^{b}	59.42^{b}	61.38^{a}	< 0.001	0.973	0.465
LAB on seed (CFU·g^{-1})	2.33E + 07^{b}	6.62E + 07^{ab}	1.71E + 08^{a}	0.0370	7.03E + 04^{b}	6.86E + 07^{ab}	1.91E + 08^{a}	0.008	0.236	3.80E + 07
LAB in water (CFU·g^{-1})	5.83E + 06	2.23E + 07	5.41E + 07	0.4510	3.33E – 01^{b}	3.30E + 07^{a}	4.92E + 07^{a}	< 0.001	0.595	1.65E + 07
Sprout length (mm)	0.89	0.76	0.71	0.6490	0.00^{c}	0.76^{b}	1.61^{a}	< 0.001	0.897	0.138
Phytic acid (%)	0.85	0.86	0.80	0.4889	–	–	–	–	0.787	0.034

$^{a, b, c, ab}$ Significant differences between groups at level 0.05; control – 0% glucose; Glu1 – 0.1% glucose; Glu 2 – 1% glucose; SEM – standard error of the mean; LAB – lactic acid bacteria; CFU – colony forming unit; phytic acid was analysed only by the effects of glucose treatments because, throughout times, it was analysed in dry seed, after soaking, and at the end of sprouting (50 h)

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vation of soaked chickpeas determined an initial number of LAB microorganisms. Before any treatment, seeds exhibited 10^5 CFU·g^{-1}, and rinse water exhibited 10^0 CFU·g^{-1}. The moisture content was also increased along with sprouting times, with the most significant at 50 h of sprouting ($P < 0.001$, Table 1). Sprouting daily with water on chickpea and mung bean seeds for 120 h increased moisture over the sprouting period (Masood et al. 2014). Moreover, Khalil et al. (2007) also discovered a similar trend in moisture content, which increased over sprouting time (up to 96 h) using chickpeas seeds (Kabuli and Desi type). There was linearly increasing moisture content, but the moisture level was lower than we observed because the sprouting system was different (no periodic water spraying). The pH of the rinse water and seeds decreased over the sprouting time, and both were significantly affected by the glucose treatments (Table 1, Figure 2).

There was also a significant increase in LAB count on the seeds ($P = 0.008$) and in the rinse water ($P < 0.001$) as sprouting progressed (Table 2). Both the pH of the rinse water and seeds were lowest in the 1% glucose treatment (3.71 and 5.52, respectively) compared to control samples (6.91 and 6.42, respectively) and pH decreased at 50 h (5.19 and 5.50 for rinse water and seed, respectively) as can be seen from Table 1. The LAB count significantly increased on the seeds but not in the rinse water. The highest LAB count was in 1% glucose treatment rinse water (10^8 CFU·g^{-1}) compared to the tap water control (10^6 CFU·g^{-1}). 0.1% glucose treatment (Glu 1) was not significantly different from the control or 1% glucose treatment (Glu 2); however, the LAB count in Glu 1 was 47.93% higher than the control sample. This result shows that glucose treatment (Glu 1 and Glu 2; $P = 0.05$) increases LAB fermentation, significantly increasing lactic acid production (Table 2). Something similar was seen regarding the LAB count on seed surfaces, 10^8 CFU·g^{-1} with 1% glucose compared with 10^7 CFU·g^{-1} for the control (Table 1). Moreover, organic acids significantly increased during the sprouting process (highest at 50 h), e.g. acetic acid ($P < 0.001$), lactic acid ($P = 0.003$), and total organic acid ($P < 0.001$) (Table 2 and Figure 3).

These results suggest a possible explanation for the connection of glucose treatment and sprouting time with LAB, resulting in pH changes and the production of organic acids. Glucose treatment supports LAB during sprouting, especially using the 1% glucose treatment. Natural fermentation of chickpea seeds for 48 h increased total LAB count in the same way as fermentation after inoculation with *Lactobacillus plantarum* (Fernandez-Orozco et al. 2008). The pH of the control samples was above 6, while the Glu 1 had a pH = 5–6. Fernandez and Berry (1989) also observed a decrease in pH (lowest value = 5.20) after 36 h of chickpea sprouting. The 1% glucose treatment decreased the pH to acidic and increased lactic acid, similar to the pH changes after natural fermentation

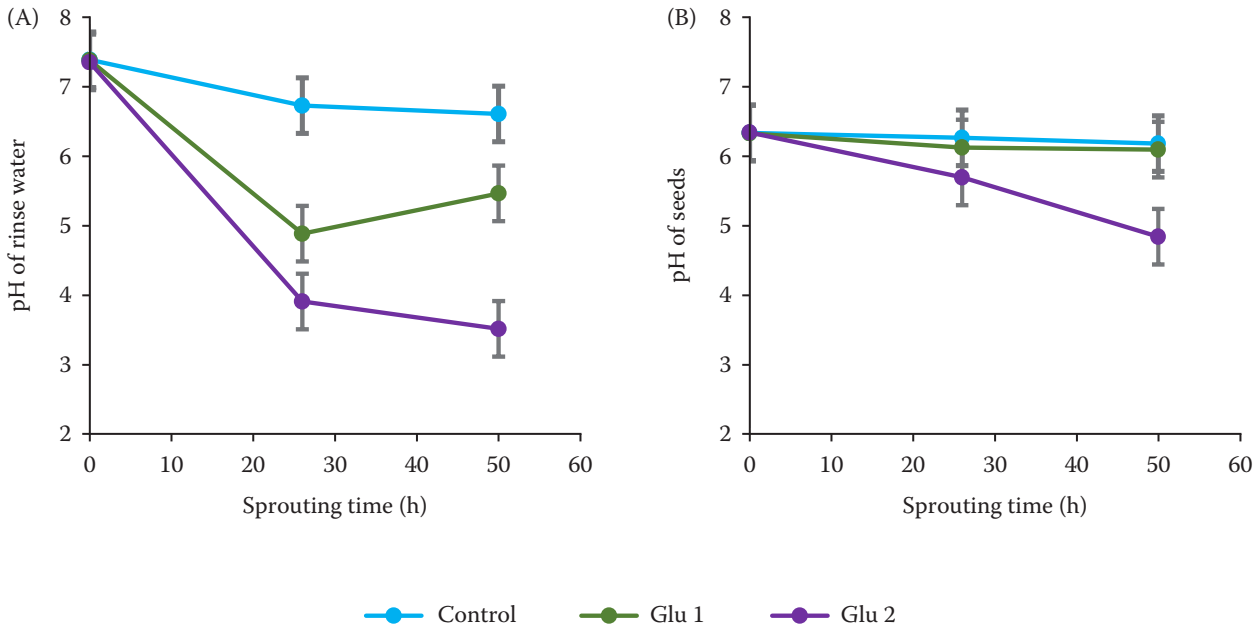

Figure 2. Changes of pH of (A) rinse water and (B) seeds throughout sprouting time at 0, 26, and 50 h

Control – 0% glucose; Glu 1 – 0.1% glucose; Glu 2 – 1% glucose

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Table 2. Significant effects and mean of glucose treatment and times on saccharides and organic acids in seeds by Kruskal-Wallis test results

Saccharides/organic acids	*N*	*df*	*P*-value	
			glucose treatment	sprouting time (h)
Glucose (%)	27	2	0.403	0.445
Fructose (%)	27	2	0.848	0.120
Sucrose (%)	27	2	0.995	0.207
Maltose (%)	27	2	0.825	0.380
Raffinose (%)	27	2	0.894	0.275
Stachyose (%)	27	2	0.979	0.168
Monosaccharides (%)	27	2	0.726	0.384
Disaccharides (%)	27	2	0.979	0.207
Oligosaccharides (%)	27	2	0.964	0.181
Total sugar (%)	27	2	0.824	0.248
Acetic acid ($mg \cdot g^{-1}$)	26	2	0.824	< 0.001*
Lactic acid ($mg \cdot g^{-1}$)	26	2	0.050*	0.003*
Total organic acid ($mg \cdot g^{-1}$)	26	2	0.550	< 0.001*

* Significant differences indicated at level 0.05; *N* – sample size; *df* – degree of freedom

of chickpeas for 48 and 72 h (4.41 and 4.25, respectively) (Dida et al. 2018). Our result showed a slightly lower pH in the rinse water (pH = 3.71), possibly due to the LAB using the added glucose to produce lactic acid and hydrolysing the sprout seed carbohydrates (Reddy et al. 2008). A pH lower than 4.5 is known to limit the growth of most food-borne pathogens (Rahman 2007).

Moreover, increased lactic, acetic acid, and total organic acid during sprouting indicate LAB fermentation of exogenous glucose and amylolytic starch (Reddy et al. 2008; Nkhata et al. 2018). Some studies of LAB or organic acids (lactic acid, acetic acid) as seed treatments during sprouting have had positive effects with implications for sprouting (Lang et al. 2000; Ding et al. 2013; Sikin et al. 2013; Budryn et al. 2019; Rossi and Lathrop 2019). Therefore, acidic conditions may promote safer sprouting. However, the seed pH is higher than the rinse water pH, which may be due to the abundant hydrogen ions in the rinse water, which contributed to the lower pH in the rinse water (Caenn et al. 2017) and the lactic acid produced by LAB in the bottom oxygen limiting part of the sprouting system.

One concern about increasing acidic conditions during sprouting is its detrimental effect on sprout growth

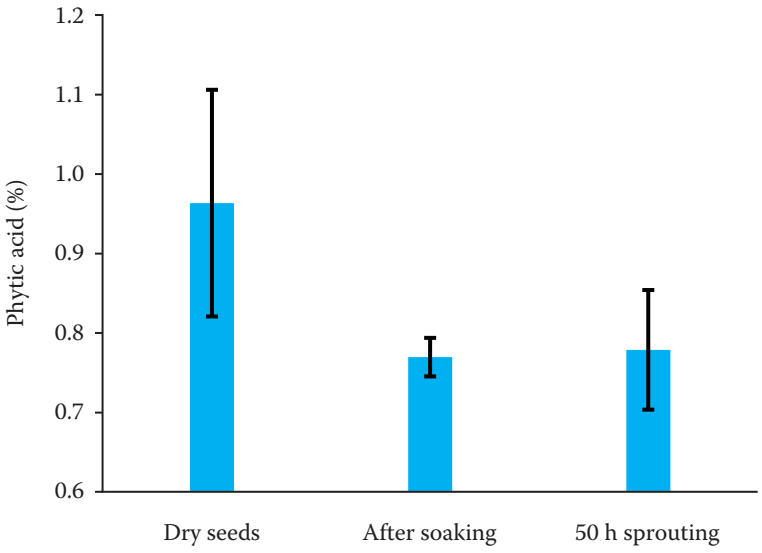

Figure 3. Phytic acid (%) in dry seeds, at 0 h (after soaking), and at 50 h of sprouting

The significant difference of phytic acid between dry seed and at 0 h and 50 h ($P < 0.001$)

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(Yang et al. 2013). However, there were no significant effects of glucose treatment on sprout length ($P = 0.649$) and an overall increase in dry mass at 50 h ($P < 0.001$) (Table 1). All sprouting groups grew; there was only a 13% and 18% decrease in dry mass in Glu 1 and Glu 2, respectively, compared to the control sample. Sprout growth can be slowed or suppressed by high acidity (low pH) stress (Sritongtae et al. 2017). A pH of 3 can decrease sprouting legumes by approximately 7%, but sprouting at pH levels of 4, 5, or 6 has similar growth (Murata et al. 2003). This range is similar to the pH at 50 h in our study, which was around 5–6.

Effects of sprouting on phytic acid and saccharides. Phytic acid was not influenced by glucose treatment ($P = 0.489$) or by the interaction of treatment and sprouting times ($P = 0.787$) (Table 1). There was a significant decrease in phytic acid between dry seeds (un-soaked) and post-soaking (time = 0 h), but no difference was found during 0–50 h of sprouting ($P < 0.001$, Figure 3). There were no significant effects of glucose treatments nor sprouting times on carbohydrates, including mono-, di-, oligo-, or total saccharides ($P > 0.05$, Table 2).

The first step of sprouting is soaking seeds in water to weaken the seed coat and allow water absorption into the cotyledon to start germination (Aloo et al. 2021; Kaur and Prasad 2021). Sprouting can improve the nutritional quality of sprout seeds by improving digestibility (Benincasa et al. 2019). Fermentation can also help reduce phytic acid to free minerals and hydrolyse oligosaccharides into more digestible sugars (Nkhata et al. 2018; Kaur and Prasad 2021).

In our study, after 24 h of soaking, phytic acid decreased by 20%, but there were no significant changes after the start of germination or up to 50 h of sprouting. This might be because the water-soluble phytic acid leached into soak water (Gibson et al. 2018); there were no changes during sprouting or fermentation. Phytic acid reduction during sprouting also depends on native phytase enzymes in seeds. Legumes like chickpeas have lower phytase activity than other seeds such as cereals (Montemurro et al. 2019). Khalil et al. (2007) found a significant decrease in phytic acid after soaking for eight hours compared to unsoaked seeds; it continued to decrease through 96 h of sprouting. Soaking for 24 h or germinating for another 24 h decreased phytic acid by around 16% (Desalegn 2015). Germination for 24 h and natural fermentation for 72 h also reduced phytic acid in chickpeas (Dida et al. 2018).

On the other hand, Egli et al. (2002) discovered that most seeds and seeds, including chickpeas, soaked for 16 h and germinated for 72 h did not effectively reduce phytic acid. It needs to be noted that many factors can play a role in this process. For instance, optimal phytate degradation is 38–55 °C at a pH of 4.5–8 in legumes (Elliott et al. 2022), while maximum phytase activity in chickpeas was observed 6–8 days after germination (Kyriakidis et al. 1998).

Germination changes the physical and chemical compositions of carbohydrates, proteins, and minerals and liberates several anti-nutrients (Nkhata et al. 2018; Aloo et al. 2021). Pulses such as chickpeas are rich in starch (55–65%) (Rahman 2007), which can be hydrolysed by hydrolytic enzymes to simple sugars (mainly glucose) and which can be used as energy during germination (Perata et al. 1998; Olaerts et al. 2016; Nkhata et al. 2018). However, the breakdown rate of the outer layer of pulses differs for different legumes and is lower when there is higher starch content. For instance, chickpea has higher hulls and flour starch than peas and lentils (Dalgetty and Biak 2003). This can also be why germinating chickpeas for up to 36 h did not change the starch structure (Fernandez and Berry 1989). Erba et al. (2018) found only a slight increase in porous structures in the starch granules of chickpeas after three days of germination. Desalegn (2015) found increased carbohydrate content in chickpea sprouts after 24 h of germination. However, germination for up to three days (after 24 h of soaking) did not significantly change simple sugars (Erba et al. 2018).

CONCLUSION

Glucose treatment improved and supported LAB growth, as indicated by acid production under oxygen-limiting conditions. Moreover, glucose addition did not suppress sprout growth or alter the chemical composition of sprouts (phytic acid and carbohydrates) compared to normal control sprouting. The treatment promotes sprouting by supporting natural LAB on seeds while maintaining normal sprouting. Glucose can be a beneficial sprouting treatment cheaper than other sprout treatments. However, antimicrobial activity against food spoilage and food-borne bacteria should be tested, and the effects on phytic acid after prolonged sprouting (> 50 h) should be further explored.

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