Microbial contamination and some chemical and physical properties of date fruits stored at room and refrigerator temperatures

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Abstract

Purpose – The purpose of this article is to investigate on changes of the microbial load and the chemical and physical properties of date fruits stored for 6 months under two different temperatures.

Design/methodology/approach – A composite sample of 100 kg date fruits from the Khalas variety, season 2019, was collected from the local market in Al Ahsa Province, Saudi Arabia, packaged in 1 kg lots, stored at room and refrigerator temperatures and the microbial contamination and the chemical and physical properties monitored over a period of six months of storage. Total bacteria, lactic acid bacteria, Enterobacteriaceae, yeasts and molds were counted and representatives of yeast and mold contaminants were identified using morphological, physiological and molecular typing techniques. Changes in the color and texture of the samples were also monitored during storage.

Findings – The yeasts detected were two strains of each of *Lachancea thermotolerans* and *Rhodosporidiobolus fluvialis* and one strain of *Cystofilobasidium lacus-mascardii*. For molds, one strain of each of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Aspergillus caespitosus* have been detected. No significant growth of these microorganisms was observed, but enough load persisted during storage that makes the samples not meeting the microbiological standards. There were significant changes in the color and texture of the fruits during storage.

Originality/value – These findings add important information that can help producers and processors to improve quality and promote marketing of date fruits, especially to international markets.

Keywords Date fruits, Storage, Microbial contamination, Color, Texture

Paper type Research paper

Introduction

Date fruit is the historic staple food of the people of the Arab Peninsula and still to this date, it maintained its importance as a food of economic and religious significance in this part of the world (FAO, 2021). The tree is grown mainly in the Arab Peninsula and in Northern Africa. The world production of date fruits in 2020 reached about 9.5mn tons; the Kingdom of Saudi Arabia produced about 1.5mn tons, making about 15.8% of the total world production (FAO, 2021). Khalas, with about 7.8 out of the 31mn trees grown in Saudi Arabia, makes one of the most important date varieties produced in the Kingdom (General Authority for Statistics, 2019). In spite of their importance as a food crop, microbiological studies on date fruits are very few. Because microbial contamination is one of the most important factors that affect food safety and quality, this study was conducted to contribute to providing

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information about the microbial contamination of date fruits and about the effect of storage at different temperatures on this contamination and on the chemical and physical properties of the fruits. Such information is of special importance for the international marketing of date fruits, where stringent microbiological standards are imposed in many countries in the world.

Materials and methods

Sample collection

Date fruits from the Khalas variety were collected from the local market of Hofuf City in September 2019, transferred to a date packing factory, washed, dried with hot air and packed under vacuum in polypropylene bags (Napco National, Saudi Arabia), then transferred to the laboratories of the Department of Food and Nutrition Sciences - College of Agricultural and Food Sciences - King Faisal University for examination and storage. Samples were stored for 6 months at room temperature (≈24°C) and in incubation (cooled incubator, Model MIR-554. SANYO Electric Co., Ltd. JAPAN) at refrigerator temperature (≈3°C).

Microbiological analysis

Counting, isolation and identification: Tests were performed at the beginning of storage and then monthly. Ten gram of pitted fruits were homogenized in 90 mL saline in a stomacher (Lab-Blender 400, Seward Medical, England) and further diluted as needed. Total bacterial count on nutrient agar (NA, Oxoid, CM0325) incubated at 30°C for 2–3 days, total yeast and mold counts on Potato Dextrose Agar (PDA, Oxoid, CM0139) incubated at 30°C for 2–3 days, lactic acid bacteria on de Man, Rogosa, Sharpe (MRS, Oxoid, CM0361) incubated in anaerobic Jars (Oxoid AG0025A) with gas generating kits (Oxoid, BR0038) at 30°C for 2–3 days, *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA, laboratorios conda s.a cat: 1092.00) incubated at 37°C for 24-48 hours. Representative colonies of yeasts and molds were picked and streaked on petri dishes to obtain pure isolates. A total of 45 yeast and 40 mold isolates were made. Yeasts were first identified using morphological and physiological characteristics including: colony form, cell form, sporulation, osmotolerance on 50% glucose and the ability to ferment glucose. Mold identification was through examining colony form and the forms of mycelia, hyphae and the fruiting bodies. Then identification of both the yeasts and molds was completed using molecular techniques. From the morphological and physiological tests, 12 yeast and nine mold isolates were chosen for molecular identification.

Molecular typing was done at the Pests and Plant Diseases Unit of the College of Agricultural and Food Sciences at King Faisal University. Fresh cultures from the yeast and mold isolates were prepared on PDA. The nucleic acids were extracted from yeasts according to Dellaporta, Wood, and Hicks (1983) and from molds according to Oregon State University (http://people.forestry.oregonstate.edu/steve-strauss/sites/peopledev). Polymerase chain reaction (PCR), two primers, the forward IT5 primer (5'- GGAAGTAAAAGTCGTAACAAGG-3') and the reverse ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the entire ITS region (White, Bruns, Lee, & Taylor, 1990). The PCR was done in a 25 µl reaction volume containing 1 µl of the fungal DNA extract (40 ng of total DNA), 2 µL $MgCl_2$, 2.5 of 10x PCR buffer,1.5 µL of 10 µM of each primer, 2.5 µl of 10 mM dNTPs, 0.4 µl of 5U Taq DNA polymerase and the reaction volume was completed to 25 µl with nuclease-free water. The PCR was conducted in a Thermal Cycler (Applied Biosystems, Life Technologies, Veriti 96-Well Thermal Cycler, Model 9902 Singapore) with initial denaturation at 95°C for 2 min, followed by 35 cycles of 95°C for 30 sec, annealing at 52°C for 30 sec and 72°C for 30 sec and finally polymerization at 72°C for 10 min. The PCR products were purified using QIAquick® PCR Purification Kit (Cat. No. 28106) according to manufacturing procedures. The DNA was mixed with loading buffer (bromophenol

blue dye) and placed in sample wells in the Agarose Gel using a Micropipette, after which the electrophoresis device was run. The DNA ladder (Biomatik Cat No. M7123) was taken and photographed under UV light with digital imaging system gel doc (Syngene Bio Imagins, IN Genius). The purified PCR products were sequenced by Macrogen Inc., (Korea), and sequencing of the purified isolates was performed in both directions using ITS5 and ITS4 primer pairs. Sequence alignments were edited by MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The isolates were then identified according to the Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi

Color analysis

The CIE color (L^*, a^* and b^* values) and the total color difference (ΔE^*) were measured as described in Aleid *et al.* (2013) using a HunterLab, Miniscan Ez-4500L color difference meter (Hunter Associates Laboratory, Inc., USA) standardized with black and green tiles. Measurements were conducted before and after packaging and after 2, 4 and 6 months of storage on ten individual fruits as replicates.

Texture profile analysis

Texture profile analysis (TPA) was performed as described in Aleid *et al.* (2013) using TAXTPlus (Stable Micro Systems Ltd., Surrey, UK). Initial speed: 0.5 mm/s, test speed: 0.5 mm/s, posttest speed: 1.5 mm/s, target mode: deformation distance: 5 mm, delay time: 5s, trigger type: auto (force), trigger force: 100 g, accessory: P/90 90\mm compression platen. Measurements were conducted before and after packaging and after 2, 4 and 6 months of storage on ten individual fruits as replicates.

Results and discussion

The Saudi standards for the microbial contamination of date fruits require testing for yeasts, molds, *Salmonella* and *E. coli* (GCC Standardization Organization, 2015). In this study, total bacterial count and lactic acid bacteria were also examined to test for the general hygienic condition of the fruits. The results showed that the date fruits coming from the field were mainly contaminated with molds and yeasts (Table 1). Lactic acid bacteria and Enterobacteriaceae (which include *Salmonella* and *E. coli*) were not detected while the total bacterial count was in the range 10^3 cfu/mL, which decreased steadily during storage to nondetectable levels (results not shown). The collected samples were contaminated with yeasts and molds at average loads of 2.5×10^2 and 2.0×10^2 cfu/g, respectively (Table 1). The loads of all of three replicates of the sample tested were in this range. This is a high load which makes these samples not meeting the stringent Saudi standard that requires loads to lie in the range of 10–10^2 cfu/g for yeasts and 10^2–10^3 cfu/g for molds in only 2 out of 5 replicates of a sample. Washing before packaging resulted in a small and insignificant reduction in the contamination with yeasts. During storage at room temperature, the loads of yeasts decreased steadily and only few colonies could be found on dishes from dilutions 10^{-1} to give average loads of approximately 10 cfu/g, which make them on the borderline of meeting the standard. The loads of the samples stored in the refrigerator increased significantly in the first month then remained almost constant during the rest of the storage period. The loads were generally high, with all of the three replicates contaminated at the range 10^2 cfu/g, which makes all samples not meeting the standard. Washing before packaging resulted in a significant reduction in the contamination with molds, but the remaining contaminants persisted without significant change during the six months of storage at room temperature. The contaminating molds did not grow but their loads were high enough to make the samples not meeting the quality standards (Table 1). The amount of contamination with molds in the

Storage duration (month)	Yeasts (cfu/g)	Molds (cfu/g)	L^*	a^*	b^*	ΔE^*
Samples stored at room temperature (≈24°C)						
Before packaging	2.5×10^{2a}	2.0×10^{2a}	40.35^{a}	17.45^{a}	19.29^{a}	–
After packaging	2.1×10^{2a}	1.1×10^{2b}	37.19^{b}	15.25^{b}	12.06^{b}	8.19^{c}
1	53^{b}	77^{c}				
2	43^{b}	1.5×10^{2b}	33.85^{c}	14.62^{b}	11.88^{b}	10.26^{b}
3	25^{c}	1.3×10^{2b}				
4	15^{c}	1.0×10^{2b}	29.93^{cd}	10.61^{c}	8.48^{c}	15.31^{a}
5	10^{c}	1.1×10^{2b}				
6	10^{c}	1.2×10^{2b}	25.83^{d}	10.58^{c}	7.70^{c}	16.84^{a}
Samples stored at refrigerator temperature (≈3°C)						
Before packaging	2.5×10^{2b}	2.0×10^{2a}	40.35^{a}	17.45^{a}	19.29^{a}	–
After packaging	2.1×10^{2b}	1.1×10^{2b}	37.19^{b}	15.25^{ab}	16.06^{ab}	8.19^{b}
1	5.8×10^{2a}	1.7×10^{2b}				
2	4.8×10^{2a}	1.4×10^{2b}	34.84^{c}	13.97^{b}	13.83^{c}	10.68^{a}
3	5.0×10^{2a}	1.1×10^{2b}				
4	6.4×10^{2a}	2.5×10^{2a}	34.52^{c}	14.85^{b}	12.15^{c}	9.86^{a}
5	4.5×10^{2a}	2.8×10^{2a}				
6	5.6×10^{2a}	2.5×10^{2a}	33.56^{c}	13.42^{b}	12.9^{c}	10.53^{a}

Note(s): No significant differences between numbers followed by same letters in a column. Values were averages of three replicates
Source(s): Table by authors

Table 1. Microbial loads and color change of date fruit samples during storage

samples stored in the refrigerator remained without significant change during the first three months to increase significantly during the last three months of storage. This amount of contamination makes the samples not meeting the quality standards. It is therefore very important to improve pre and postharvest hygienic conditions to control this microbial contamination and reduce it to acceptable levels. Al Jasser (2010) reported loads of osmophilic yeasts in date fruits at 10^2 cfu/g, which persisted in approximately the same range for 6 months at both room and refrigerator temperatures. Aleid *et al.* (2014) and Hamad (2012) reported date fruit contamination with yeasts and molds in the ranges 10^2–10^3 cfu/g. Zamir *et al.* (2018) reported loads up to 5.65 Log cfu/g aerobic bacteria and up to 5.36 Log cfu/g yeasts and molds and no coliforms nor *E. coli* in date fruit samples collected from local outlets in Dhaka City, Bangladesh. Samples of date fruits collected from different locations in Morocco were found contaminated with bacteria and yeasts and molds at loads of up to 4.2 log cfu/g and up to 2.99 cfu/g, respectively, and no contamination with coliforms, *Bacillus* sp. and *Staphylococcus* (Jdaini *et al.*, 2022). Piombo *et al.* (2020) detected *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria and Candida* in Medjool date fruits sampled in the southern Arava region, Israel. Taouda, Aarab, and Chabir (2018) detected loads of molds at log 0.6 to log 3.8 cfu/g and of yeasts and total bacteria at log 0.6–log 4.9 cfu/g in 13 different varieties of date fruit samples collected from Fez city of Morocco. Al Jawally (2010) found 53 out of 245 (21.6%) date fruit samples collected from local markets, retailed products and processed by-products from factories in Abu Dhabi Emirate not compliant with UAE regulations due to total count of osmophilic yeast and mold exceeding the maximum limit according to microbiological criteria in the UAE standard No.: 1016/2002, (2). Al Hazzani *et al.* (2014) described *Aspergillus niger, Staphylococcus aureus, Escherichia coli and Bacillus* spp. as the dominant contaminants of date fruit samples collected from markets in Riyadh, Medina and Kharj, Saudi Arabia. According to Abass (2013), the dominant microbial contaminants of Iraqi date fruits are the fungi *Aspergillus niger, Alternaria alternata* and *Penicillium* and the bacterial genera *Bacillus, Staphylococcus* and *Proteus*.

A significant change in the color of the fruits occurred, especially in the samples stored at room temperature as indicated by a significant increase in ΔE^* (Table 1). This is in agreement to results reported many researchers (Hazbavi, Khoshtaghaza, Mostaan, & Banakar, 2013; Ruenroengklin *et al.*, 2008) for date fruit samples stored for 6 months at 25°C. Color change in the samples stored in the refrigerator was smaller; this could be due to slowed degradation reactions of color pigments under the low temperatures. The color of the samples became darker probably due to oxidation of polyphenols during storage. The decomposition of polyphenols and tannins leads to a decrease in the degree of lightness (Sant Anna *et al.*, 2013), noting that anthocyanins, tannins and polyphenol oxidase are present in date fruits (Al Khatib & Dinar, 2002). The original color of the date fruits under study was more red than green and more yellow than blue with positive a^* and b^*values, respectively. These shades of color changed toward green and blue as both a^* and b^* decreased significantly during storage (Table 1). This could be caused by the degradation of the reddish anthocyanin and the yellowish flavonoids present in date fruits. Carotenoids are sensitive to light, pH, heat and oxygen (Limbo, Torri, & Piergiovanni, 2007). Al Alawi *et al.* (2017) reported that Khalas fruits contain the highest amount of carotenoids compared to other date fruits.

Packaging and storage affected the texture of the date fruits (Table 2). Hardness of the fruits (measure of the force required for deformation) decreased significantly after packaging and during storage, especially in the samples stored at room temperature. Springiness: the rate at which a deformed sample returns to its original size and shape, decreased significantly while adhesiveness; the negative force area for the first bite, representing the work required to overcome the sticky forces between the sample and the probe; increased significantly at both storage temperatures. Cohesiveness: the strength of internal bonds in the sample; chewiness: the energy needed to chew a solid food until it is ready for swallowing; and resilience: a measure of how well a product fights to regain its original position remained generally unchanged. Date fruits contain polygalacturonase, pectinesterase and cellulase enzymes, which can breakdown pectins and cellulose in the fruit and hence contribute to changes in its texture (Kamal-Eldin *et al.*, 2020; Požrl *et al.*, 2010; Toivonen & Brummell, 2008; Barreveld, 1993).

Storage (months)	Hardness (g)	Adhesiveness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Resilience (ratio)
Samples stored at room temperature (≈24°C)						
Before packing	4113.1^{a}	−0.17^{b}	0.56^{a}	0.39^{a}	395^{a}	0.09^{a}
After packing	1855.2^{b}	−0.15^{b}	0.51^{a}	0.39^{a}	365^{a}	0.07^{a}
2	1062.0^{c}	−0.96^{a}	0.48^{b}	0.39^{a}	263^{b}	0.07^{a}
4	806.7^{d}	−0.86^{a}	0.31^{c}	0.33^{a}	336^{a}	0.09^{a}
6	572.0^{e}	−0.75^{a}	0.28^{c}	0.40^{a}	307^{a}	0.10^{a}
Samples stored at refrigerator temperature (≈3°C)						
Before packing	4113.1^{a}	−0.17^{b}	0.56^{a}	0.39^{a}	395^{a}	0.09^{a}
After packing	1855.2^{b}	−0.15^{b}	0.51^{a}	0.39^{a}	365^{a}	0.07^{a}
2	873.0^{c}	−1.45^{a}	0.39^{b}	0.37^{a}	214^{b}	0.07^{a}
4	861.3^{c}	−1.38^{a}	0.29^{b}	0.34^{a}	353^{a}	0.10^{a}
6	793.0^{d}	−1.31^{a}	0.31^{b}	0.41^{a}	299^{ab}	0.10^{a}

Note(s): No significant differences between numbers followed by same letters in a column. Values were averages of three replicates
Source(s): Table by authors

Table 2. Texture changes of date fruit samples during storage

Identification of the microbes isolated from the date fruit samples

The results of yeast and mold identification are shown in Table 3.

Yeasts were detected in date fruit samples before and after packaging and during the storage period, where three genera of yeasts were identified, namely *Lachancea thermotolerans*, *Rhodosporidiobolus fluvialis* and *Cystofilobasidium lacus-mascardii*. The prevailing yeast directly after packaging was *Lachancea thermotolerans*, making about 50% of the total yeasts population contaminating the samples. Following was the yeast *Rhodosporidiobolus fluvialis* with about 30% then the yeast *Cystofilobasidium lacus-mascardii* with about 20% of the yeasts contaminating the samples.

Lachancea thermotolerans was also dominant during storage. It made about 90% of the yeast population of the samples after one month of storage at both temperatures. Its presence decreased to about 50% and 70% of the population by the end of the six-month storage period at room and refrigerator temperatures, respectively. This yeast, formally called *Kluyveromyces thermotolerans*, is psychrophilic with maximum growth temperature of some of its strains of about 30°C (Barnett, Payne, & Yarrow, 2000). Two different strains were isolated from this yeast; one was dominant making about 85% of the isolates. This yeast, being psychrophilic, able to ferment glucose and fructose and osmophilic growing in 60% glucose, can be considered as one of the most important date fruit contaminants, especially fruits packed and stored under refrigeration. It is used in the wine industry to produce flavors, combat spoilage and improve

Table 3.
Yeasts and molds isolated from the date fruits samples under study

Isolate	Colony color	Growth on 50% glucose	Glucose fermentation	Sporulation	Name of microbe
1	White	+	+	+	*Lachancea thermotolerans*
2	White	+	+	+	*Lachancea thermotolerans*
3	White	+	+	+	*Lachancea thermotolerans*
4	White	+	+	+	*Lachancea thermotolerans*
5	White	+	+	+	*Lachancea thermotolerans*
6	White	+	+	+	*Lachancea thermotolerans*
7	Pale pink	+	−	+	*Cystofilobasidium lacus-mascardii*
8	Pale pink	+	−	+	*Cystofilobasidium lacus-mascardii*
9	Pale pink	+	−	+	*Cystofilobasidium lacus-mascardii*
10	Pink	+	−	+	*Rhodosporidium fluviale*
11	Pink	+	−	+	*Rhodosporidiobolus fluvialis*
12	Pink	+	−	+	*Rhodosporidiobolus fluvialis*
13	Brown to green	n.d	n.d	+	*Aspergillus caespitosus*
14	Yellow to green	n.d	n.d	+	*Aspergillus flavus*
15	Yellow to green	n.d	n.d	+	*Aspergillus flavus*
16	Yellow to green	n.d	n.d	+	*Aspergillus flavus*
17	Bluish green	n.d	n.d	+	*Penicillium chrysogenum*
18	Black	n.d	n.d	+	*Aspergillus niger*
19	Black	n.d	n.d	+	*Aspergillus niger*
20	Black	n.d	n.d	+	*Aspergillus niger*
21	Black	n.d	n.d	+	*Aspergillus niger*

Note(s): (+): positive result (−): negative result, n.d.: not done
Source(s): Table by authors

color (Morata *et al.*, 2018). Some of its strains are also used in the biocontrol of molds in grapes, especially to control spoilage by *Aspergillus* (Morata *et al.*, 2018). Since this fungus is a significant contaminant of date fruits, this yeast can be considered for its biocontrol.

The presence of *Rhodosporidiobolus fluvialis*, which was called *Rhodosporidium fluviale* (Wang *et al.*, 2015), in samples stored at room temperature decreased from about 30% of yeast population at the beginning of storage to disappear in the second month of storage. Contrarily, it persisted in the samples stored at refrigerator temperature at about 30–20% of the yeast population during the storage period, indicating that it is an important contaminant of date fruits stored under refrigeration. This yeast is psychrotrophic with maximum growth temperature of 37°C. Although some of its strains are not osmophilic, showing no growth at 50% glucose concentration (Barnett *et al.*, 2000), but the two strains isolated in this study were osmophilic showing good growth at 50% glucose concentration. The two strains of this yeast were present at about 67% and 33% of its isolates. This yeast is used in the production of biodiesel, with some of its strains producing more than 20% of their dry weight as lipids (Polburee *et al.*, 2015). In addition, some strains are used to control fungi that cause fruit spoilage, such as *Botrytis cinerea* in apples (Sansone *et al.*, 2018).

Cystofilobasidium lacus-mascardii, represented by only one strain, was present in the samples stored at room temperature but at very low concentrations toward the end of the storage period. It was also present in the sample stored in the refrigerator but at a low percentage of about 10% of the yeast population at the end of the storage period. The yeast is psychrotrophic, with maximum growth temperature for some strains of 25°C (Libkind, Gadanho, Van Broock, & Sampaio, 2009), does not ferment glucose and is not osmophilic with no growth at 50% glucose concentration. With these characteristics, this yeast can be looked at as a contaminant of minor importance for date fruits.

The genera and species of molds detected in the date samples under study were: *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus flavus* and *Aspergillus caespitosus* (Table 3). All of these genera and species were reported as date fruit contaminants by Al Asmari *et al.* (2017), Hamad (2008) and Hasnaoui *et al.* (2010). *Aspergillus niger* was detected in the samples before and after packaging and during storage at room and refrigerator temperatures, making 100% to about 88% of the mold population contaminating the fruits throughout this period. This fungus is widely spread in nature, enduring wide range of temperature, pH and water activity, a fact that explains its abundant presence as contaminant of date fruits whether stored in refrigeration or at room temperature (Astoreca *et al.*, 2007; Schuster *et al.*, 2002). All of the isolates identified in this study belonged to one strain. *A. niger* is generally recognized as safe (GRAS) according to the US Food and Drug Administration, and it has been used commercially for decades for the production of citric acid and a group of enzymes. However, some of its strains that contaminate food may cause allergies and even liver cancer (Gautam *et al.*, 2011; Cairns, Nai, & Meyer, 2018). Since this fungus was found in the date fruits under study in all stages of storage and in relatively large quantities, the potential toxicity of strains contaminating date fruits should be taken into consideration.

The potential pathogen *Aspergillus flavus* was detected in the date fruit samples before and after packaging and in the samples stored at refrigerator temperature but not in the samples stored at room temperature. It was present at low levels making only about 4–12% of the mold population contaminating the fruits. The isolates identified belonged to one strain. This fungus is the producer of the famous aflatoxins; it is also known to cause the spoilage of up to 25% of food crops worldwide (WHO, 2018).

Aspergillus caespitosus was found in the samples before and after packaging and during storage at the refrigerator temperature only. Its highest presence did not exceed 4% of the population of the molds contaminating the samples. This fungus is not considered a human pathogen, but was isolated from a patient with tuberculosis, but it was not known whether it was the cause of the infection or not (Salah *et al.*, 2019).

Penicillium chrysogenum was detected in the samples before but not after packaging neither during storage. The numbers were very limited and did not exceed 5% of the mold population contaminating the samples. Although this fungus is safely used for the commercial production of drugs, some of its strains may cause skin and respiratory diseases especially to people with low immunity (Aviles *et al.*, 2016; Elander, 2003).

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