

# Sugary Foods and Beverages Relationship to Fungal Colonization and Oral Hygiene in School Children

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Received: 15.12.2021; Accepted: 20.01.2022; Published: 18.03.2022

**Abstract:** Sugary foods and beverages are highly consumed by children. It is a substrate for fungal growth and has effects on oral hygiene. The study aimed to determine sugary foods and beverages' relationship to oral fungi and hygiene. This cross-sectional study was conducted on 150 students aged 11-12 years old, selected based on inclusive-exclusive criteria. Demographic data and sugary foods and beverages consumption were recorded in a questionnaire. The oral material was collected and cultured in SDA media. Fungal growth was evaluated microscopically, and then fungal identification used the Integral System YEASTS Plus (Liofilchem®). OHI was used to assess oral hygiene. Results showed that 72.0% were 12 years old, the frequency of sugary foods and beverages consumption was more than once per week in 85 children (56.67 %), mostly girls (30.9%). The fungus found was mostly *Candida* (55.3%), and 43.3% of children had poor oral hygiene. Statistical analysis showed a significant association between the frequency of sugary foods and beverages consumption with fungal colonization ( $p < 0.05$ ) and oral hygiene ( $p < 0.05$ ). The correlation between fungal colonization and oral hygiene was statistically significant ( $p < 0.05$ ). This study provides insight into the relationships between sugary foods and beverages, fungal colonization, and oral hygiene.

**Keywords:** sugary foods and beverages; oral hygiene; fungal colonization.

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## 1. Introduction

Nowadays, people's lifestyles tend to adopt an "unhealthy" behavior [1]. In this case, school-age children are an interesting sub-population to study because of the high burden and demand of subjects in primary school and its associated high intense stress levels. This condition further facilitates unhealthy behavior, such as a tendency to overeat and consume foods and beverages high in sugar [2, 3]. More than half of children consumed sugary foods and beverages occurred at home. Thus, the family's food eating environment and parental behavior may influence children's behavior in consuming sugary foods and beverages [2,4].

Improper eating habits, such as the high consumption of sugary drinks, can cause many health problems [5-7]. One study found the drinks most in-demand and consumed by children and adolescents are those sweetened with sugar [8].

Sugar-rich foods and drinks are excellent substrates for the microorganisms that inhabit the early parts of digestion [9, 10], and excess sugar can cause an overproduction of fungi. A study in China reported that 21.7% of Chinese children received more than one portion of sweet foods and drinks per week, while 9.5% received one or more portions of sugary foods and drinks per day [11]. Daily consumption of sugar had exceeded recommended sugar intake

among adolescents and declined through adulthood [12]. In addition, some researchers have reported that consuming excessive sugar decreases the pH of the oral cavity and changes the microenvironment [7,13].

The oral cavity is one of the most colonized parts of the human body [14, 15]. The prevalence of fungi in humans increases, estimated to range from 2% to 70% in the immunocompetent population and immunocompromised people about 96% [16]. Poor oral hygiene is often considered a predisposing factor for oral candida colonization, and the species most frequently isolated is *Candida albicans* [17-19]. *Candida albicans* is a commensal fungus commonly found in the oral cavity of 18,5-40,9% of healthy people [19]. Other *Candida* species, such as *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. krusei*, and *Kluyveromyces marxianus*, have rarely been isolated, but their prevalence has increased over the last decade [20]. *Candida* is an opportunistic-pathogen, commensal and normally found in the oral cavity and found more frequently in younger children because the immune system in children is still in the development stage [19,21].

Current research focuses on finding the relationship between sugary foods/drinks consumption and their impact on nutritional status, cardiovascular system, digestive tract microbiota, obesity, and obesity-related diseases. There is evidence that the consumption of sugary foods and beverages is related to the oral ecosystem. However, the relation between the frequency of sugary foods and beverages consumption and oral microorganisms of school children and early adolescence is still rarely studied. This study objective was to determine the relationships between the frequency of sugary foods and beverages consumption, oral fungal colonization, and oral hygiene of school-age children.

## 2. Materials and Methods

### 2.1. Study design.

The study was cross-sectional, and the recruitment of subjects used a consecutive sampling technique based on the inclusion criteria. The students who participated in the study were 150 students aged 11-12 years from a public primary school in Gambir sub-district, Central Jakarta. The children must have parental consent in the study. Signed informed consent from parents was requested. This study had ethical clearance from the Medical Research Ethics Commission of Universitas Trisakti, which reviewed and approved the research protocol.

Demographic information and consumption of sugary foods and drinks for one week were examined using the modified questionnaire of Neuhouser *et al.* [22]. Oral hygiene status was determined after examination of oral conditions and was assessed using the Simplified Oral Hygiene Index (OHI-S), which summed the Debris Index and Calculus Index. Each was determined from the amounts of debris and calculus on the six tooth surfaces [23].

### 2.2. Sample collection and direct microscopic examination.

The tools and materials used are sterile distilled water, beaker glass, phosphate buffer solution, solid Sabouraud's Dextrose Agar (SDA) media, object-glass, inoculum loop, bunsen burner, agar plate, LPCB, microscope.

Procedure: All the respondents were asked to thoroughly rinse their mouths with 100 ml of distilled water to remove any food debris. After 10 min, phosphate buffer solution was used as an oral rinse method for saliva collection. Samples were obtained by requesting

respondents to keep and swirl the solution for 1 min, and then expectorate all saliva into pre-sterilized container without swallowing. Then the saliva was carried with a pipette and inoculated in Sabouraud's Dextrose Agar media. The sample was streaked using inoculating loop and incubated at 37°C for 48 hours. The growth appeared in 48 hours as cream/white-colored, smooth, and pasty colonies. Fungal growth was evaluated using a microscope with LPCB staining, positive culture then was transmitted on solid media Sabouraud's Dextrose Agar (SDA) to obtain pure isolates.

### 2.3. Isolation and species identification.

Furthermore, the obtained fungi were identified using a commercial kit (Liofilchem® Integral System YEASTS Plus, cat. no. 7182279822, Italy), a 24 reaction well panel containing biochemical substrate and antimycotics for the identification of the most clinically important yeasts. This tool also includes a chromogenic well (no. 13-CHR) which delivers a color-based distinction: green color for *Candida albicans*, and purple color for *Candida tropicalis*. The panel is inoculated with the cell suspension, and identification results are delivered after incubating at 36°C for 48 hours (Fig. 1B) [24]. The relationship between consumption of sugary foods and drinks to the risk of mold in the oral cavity was then determined.

### 2.4. Procedure of oral hygiene examination.

The instruments and supplies required for the oral examination included plane mouth mirrors; metallic periodontal probes; containers and concentrated disinfecting solution; rubber gloves; washbasin for water and soap or disinfectant solution; cloth or paper hand towels; and gauze. Used instruments should be placed in a disinfectant solution, then washed and drained well before sterilization. The area for conducting examinations should be arranged for optimum efficiency. The lighting should be consistent throughout the examination. If necessary, examinations can be carried out outside.

The Oral Hygiene Index is composed of the combined Debris Index and Calculus index; each of these indexes is in turn based on 12 numerical determinations representing the number of debris or calculus found on the buccal and lingual surfaces of each of three segments of each dental arch, namely distal segment to the right cuspid, distal segment to the left cuspid, and mesial segment to the right and left first bicuspid. The maxillary and the mandibular arches are each composed of three segments. Each segment is examined for debris or calculus. From each segment, one tooth is used for calculating the individual index for that particular segment. The tooth used for the calculation must have the greatest area covered by either debris or calculus. The method for scoring calculus is the same as that applied to debris.

#### Debris Index Criteria

0 = No debris or stain present.

1 = Soft debris covering not more than one-third of the tooth surface or presence of extrinsic stains without other debris regardless of surface area covered.

2 = Soft debris covering more than one-third, but not more than two-thirds, of the exposed tooth surface.

3 = Soft debris covering more than two-thirds of the exposed tooth surface.

#### Calculus Index Criteria

0 = No calculus present.

1 = Supragingival calculus covering not more than a third of the exposed tooth surface.

2 = Supragingival calculus covering more than one-third but not more than two-thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.

3 = Supragingival calculus covering more than two-thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both. The oral hygiene index is the sum of the debris index and calculus index (Oral Hygiene Index = Debris Index + Calculus Index).

### 2.5. Statistical analysis.

The data were statistically analyzed using the Chi-square test to identify relationships in the data obtained in the survey and from the fungal identification and OHI test results. Statistical analyzes were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) software with a 95% confidence level ( $\alpha = 0.05$ ). The results were considered statistically significant at a p-value  $< 0.05$ .

## 3. Results and Discussion

The number of respondents' gender in this study was not much different; namely, 54.0% (81/150) girls and 46.0% (69/150) boys, and most of the respondent's age was 12 years (108/150; 72.0%), and the rest was aged 11 years (42/150; 28.0% - Table 1). The result of this study showed that 56.67% (85/150) of respondents consumed sugary foods and drank more than once per week ( $\leq 1$  time per week), and 65/150 (43.33%) had consumed sugary foods and drank less than once per week ( $< 1$  time per week).

**Table 1.** Characteristics of respondents, sweet foods, and drinks.

Category	Frequency	Sugary Foods and Drinks Consumption		p-Value
	n (%)	$< 1x/week$ (%), n=65	$\geq 1x/week$ (%), n=85	
Age				
11 yo	42 (28.0)	31 (20.7)	12 (8.0)	0.000 <sup>a</sup>
12 yo	108 (72.0)	20 (13.3)	87 (58.0)	
Gender				
Male	69 (46.0)	26 (17.1)	38 (25.0)	0.000 <sup>a</sup>
Female	81 (54.0)	39 (25.7)	47 (30.9)	
Colony				
Candida Unidentified	83 (55.3) 67 (44.7)	41(27.3) 23 (15.3)	42(28.0) 44 (29.4)	0.000 <sup>a</sup>
OHI				
Good	85 (56.7)	39(26.0) 26 (17.3)	46(30.7) 39 (26.0)	0.000 <sup>a</sup>
Poor	65 (43.3)			

<sup>a</sup>Chi-square test,  $p < 0.05$ .

All collected samples were directly streaked on SDA. Of the 150 samples, 55.33% were yeasts in 83 isolates, and 44.67% were unidentified in 67 isolates (Table 1). The colonies' growth was cream/white colored colonies, protruding from the surface of the medium; the colony's surface was smooth and pasty (Fig.1A). Candida species identification used Integral System YEASTS Plus that showed a chromogenic well (no. 13-CHR) changing color into green, indicating *Candida albicans* (Fig.1B). The proportion of respondents with a good Oral Hygiene Index (OHI) was 56.7%, and 43.3% had poor OHI.

This study found that respondents aged 12 years who consume sugary foods and beverages more than once per week were 58.0% (87 children), and 30.9% (47 children) were girls. Statistical analysis showed that the age and gender of respondents were significantly associated with sugary foods and beverages consumption ( $p < 0.05$ , Chi-square test).



**Figure 1.** (a) Colonies of *Candida* on solid media Sabouraud's Dextrose Agar; (b) Identification of *Candida albicans* with Integral System YEASTS Plus.

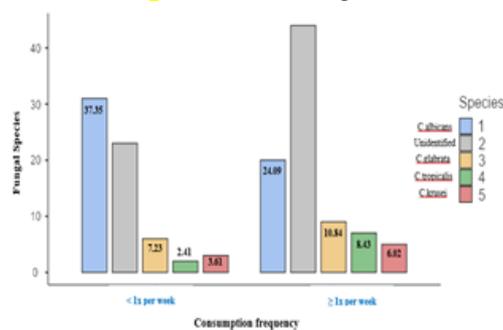
Previously, it has been known that behavioral differences connected with caries are related to gender, for instance, girls generally maintain better oral health by brushing their teeth more regularly, but they also consume more sugary foods than boys [25]. In general, the age of 12 is considered an important period in developing dental caries and oral health impairment [26]. The consumption of high amounts of simple carbohydrates can increase the risk of dental caries because the cariogenic microorganisms require sugar for their nutrition and protect oral health [27, 28]. The frequency of sugar intake is responsible for changing the balance of the microorganism's population. In addition, the acidity of the oral cavity is a prerequisite for caries formation, and acidogenic microorganisms play an important role in oral hygiene [29, 30]. Thus, it is recommended for children and adults to reduce free sugars intake to 10% of total energy intake [31, 32].

One study reported that 50.9% of 915 respondents were boys at 10 years follow-ups, 52.7% of 996 respondents were girls at 15 years follow-ups [29], and Guo *et al.* [33] also found that 51.0% of respondents were boys at 6-11 years. He *et al.* [11] reported that age and gender were associated significantly with the frequency of sugary foods and beverages consumption. In a study involving 2,032 respondents consisting of 1,019 girls (50.15%) and 1,013 boys (49.85%), with the age of respondents ranging from 6 to 12 years, as many as 69.0% consumed sugary drinks. They also found that 34.7% of respondents did not consume sugary drinks, and 21.6% consumed sugary drinks of more than 120 ml per day (equivalent to more than 3 times per week), mostly in girls. Similar to the finding in New Zealand, involving 578 school-age children found 313 girls (54%) with an age range of 8 to 12 years, and 16.7% of respondents consumed sugary beverages once a day or more per week they were also girls [34]. Another study reported that 9.6% of respondents consumed sugary beverages more than 7 portions per week [35]. In Germany found, high daily consumption of sugary beverages was more than 4 times in girls aged 3 – 17 years and more than 6 times in boys [36]. Based on previous studies, it is known that children aged 11-12 years often consume sugary foods and more in boys, while in this study was more in girls. This may be related to various factors, e.g., parenting patterns and puberty period in some girls start at age 11-12 years. The literature suggests that hormones and stress can affect the increase in consumption of sweet foods in women.

Fungal colonization in this study was identified in respondents with consumption frequency of sugary foods and beverages more than once a week (28.0%) and less than once a week (27.3%) had a similar proportion (Table 1). Furthermore, fungus grew on all culture media and mostly was *Candida* species; due to the commercial kit's limitations, 67 fungus

isolates could not be identified. *Candida* species that were found in respondents who consume sugary foods and beverages more than once a week consisted of *Candida albicans* (24.09%), *Candida glabrata* (10.84%), *Candida tropicalis* (8.43%), *Candida krusei* (6.02%), and 44 isolates (53.01%) were unidentified (Fig.2). Fungal colonization was significantly increased in respondents who consumed sugary foods and beverages ( $p < 0.05$ , Table 1).

The oral microbiota can cause various oral health interference, and *Candida* species can lead to uncontrolled proliferation, one of which depends on diet. Consumption of sugary foods and drinks is a predisposing factor for fungal colonization. *Candida* species are opportunistic pathogens that are common inhabitants of the normal oral microorganisms, and *Candida albicans* is a very common fungal species in the oral cavity [37-39]. A previous study reported that fungi incidence in the oral cavity was statistically significant ( $p = 0.004$ ) in those adding sugar to beverages [40]. Another study demonstrated that 93,8% of respondents were positive for *Candida* spp. and *Candida albicans* was the most dominant species 97,5%, while *Candida krusei* 40%, *Candida tropicalis* 17,5%, and *Candida glabrata* were found to be 7,5% [41].



**Figure 2.** Prevalence of oral fungal species to the frequency of sweetened foods and beverages consumption.

Respondents in this study who had a frequency of sugary foods and beverages consumption more than once a week and had good OHI were 30.7%, and 26.0% had poor OHI. Oppositely, 26.0% of respondents with a frequency of sugary foods and beverages consumption less than once a week had good OHI, and 17.3% had poor OHI. The Oral Hygiene Index showed significantly associated respondents who consumed sugary foods and beverages ( $p < 0.05$ , Table 1).

**Table 2.** Characteristics of respondents, sweet foods, and drinks.

Fungal colony	Oral Hygiene Index				p-Value
	Good		Poor		
	n	%	n	%	
<i>Candida spp</i> Unidentified	49	32.67	34	22.67	0.044 <sup>a</sup>
	36	24.00	31	20.66	
Total	85	56.67	65	43.33	

<sup>a</sup> McNemar test,  $p < 0.05$

Table 2 showed that *Candida* species were found on 34/65 (22.67%) of respondents with poor oral hygiene, and respondents with good oral hygiene were 49/85 (32.67%). Based on Table 2, it could be concluded that the association between Oral Hygiene Index and oral fungal colonization was statistically significant ( $p = 0.044$ ).

The acidity condition of the oral cavity and the microorganism in the oral cavity are factors of caries formation which play an important role in oral hygiene [28,30]. It has been known previously that fungi constitute a small part of the salivary oral microbiome, with *Candida albicans* being the most dominant species [42].

Previous studies observed that children with a sugary food diet were found to have more caries and identified *Candida albicans* ranged from 12% to 93% in children with caries

[31,43]. The study of Wu *et al.* [44] showed the decrease of salivary s-IgA level correlated with caries in children was statistically significant. In contrast, Hemadi *et al.* [45] found a weak association between *Candida spp.* in the oral cavity and caries levels in children. In addition to dietary habits, consuming sugary foods and beverages has a role in regulating the ecological balance of oral microorganisms, thus being responsible for changes in oral hygiene [46, 47].

#### 4. Conclusions

This study provides additional insight into the relationship between sugary foods and beverages consumption with fungal colonization and oral hygiene in primary school children.

This study showed that sugary foods and beverages consumption has a strong association with demographic factors (age and gender). Our findings were that high intake of sugary foods and beverages consumption was associated with oral fungal colonization. Furthermore, sugary foods and beverages consumption can be one of the factors that play a role in oral hygiene due to its significant relationship with fungal colonization ( $p < 0.05$ ) and OHI ( $p < 0.05$ ).

The consumption of sugary food and beverages in children should be controlled by promoting a healthy lifestyle in school. In addition, counseling is highly recommended to change the lifestyle of the families, especially high-risk groups, which may help improve dental and oral health in children.

#### Funding

This research received no external funding.

#### Acknowledgments

This study was funded by a grant from the Medical Research Council, Universitas Trisakti (No. 053/A.5/LPT/USAKTI/I/2018).

#### Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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