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FUNGI CONTAMINATION OF DRIED FRUITS SOLD WITHOUT PACKAGING IN THE CONTEXT OF WATER ACTIVITY, WATER CONTENT AND PH

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Summary. Dried fruits can be packed or sold without packing, what implicate the different extrinsic factors influencing microbial growth. The hygienic conditions prevailing at the point of sale, time and manner of exposure as well as quality of air could affect the microbial quality of that products.When the relative humidity and moisture content balance is disturbed, a suitable moisture environment is created especially for mold growth. The examined products were characterized by proper physicochemical parameters, which resulted in low contamination with mold microflora. A simple mathematical model was proposed to describe the dependence of water activity on water content of dried cranberries and raisins sold without packaging. The value of estimated coefficient of determination indicated a weak dependence of water activity a_w on water content examined products. The microbiological analysis of samples of cranberries and raisins showed slight contamination by the fungal microflora – mold and yeast. The lower microbial load of cranberry samples correlated with their pH and the natural presence of compounds with antimicrobial activity.

Key words: raisins, dried cranberries, molds contamination, water activity, water content, pH

INTRODUCTION

Fruits are an important part of a healthy and balanced diet in all societies around the globe. They are rich in carbohydrates and many vitamins, proteins, minerals and dietary fiber. The microbial contamination usually results from contact with dust, soil, and wastewater during harvest and post-harvest periods. Fresh fruits are highly perishable due to their high water content of about 80%. Drying is one of the oldest and most common

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methods of processing and preserving food. The water activity (a_w) of dried fruits and vegetables is generally ca.0.70 what prolongs their shelf life [Hui and Barta 2006]. The FAO/WHO define low moisture foods (LMF) as foods that are naturally low in moisture content or are produced from higher moisture foods through drying or dehydration processes [FAO/WHO 2014].

The value of global dried fruit market amounted to USD 7.4 billion in 2020. In 2019/2020 production of dried fruit worldwide was about 3.2 million metric tons [Statista – The Statistics Portal]. Large fruits are sliced or chopped to increase surface-area to volume ratio to facilitate the loss of moisture during drying, while small such as grapes or berries can be dried as whole. The raisins are extensively consumed around the world including Poland in raw form, or in the baking and brewing industries. They have attractive flavour, texture, and they are rich in nutrients. In most cases the beneficial effects of raisins have been assessed in intervention studies focused on the cardiovascular area, diabetes, and oral health [Jeszka-Skowron et al. 2017]. The top five countries in raisin production are Turkey (about 2.95×10^5 metric tons), USA, China, Iran, and Uzbekistan [Wang et al. 2021]. Cranberries are polyphenolic-rich berry fruits offering important health benefits [Avorn et al. 1994, Nowack 2007] such as prevention from i.e. urinary tract infections, reduce the risk of cardiovascular diseases and selected types of cancer, and might have antimicrobial, antianemic, detoxifying properties [Blumberg et al. 2013]. Taking into account the various beneficial effects of cranberries on human health, consumption of these fruits is recommended. Polish climatic conditions meet the requirements for growth of large-fruited cranberries *(Vaccinum macrocarpon* L.*)*. Cranberries are also grown in the Baltic countries (Lithuania, Latvia, and Estonia) and Belarus. However, the bitter and astringent taste of cranberries limits consumption of the raw fruits. It prompts the usage of an osmotic dehydration in sugar solutions to enrich cranberries with a sweet taste and to partially remove water from the tissue [Wiktor et al. 2019]. Traditional osmotic dehydration of fruits is mainly carried out in sucrose or glucose hypertonic solutions. Another approach involves using sugar substitutions such as steviol glycosides, which decreases the energy value of osmodehydrated products [Oliveira et al. 2012]. Dried cranberries are used as an addition to bars, bran, cookies and muesli.

The growth of microorganisms in dried foods is largely prevented by high temperature used during processing and the final low water activity. Molds can survive low water activity [Beuchat 1983]. However, since dry foods are hygroscopic and the moisture content is not constant, the relative humidity in the air in the storage is important. When the relative humidity and moisture content balance is disturbed, a suitable moisture environment is created especially for mold growth. Dried fruits can be packed or sold without packing, what implicate the different extrinsic factors influencing microbial growth. After drying, fungi spores may be present, and they can remain viable even for months [Beuchat and Mann 2014].

Our aim was to analyse the fungal microflora in correlation to physicochemical parameters of dried fruit sold without packaging.

MATERIALS AND METHODS

Market analysis. An analysis of the availability (July, 2020) of dried fruit and vegetables products sold without packaging on the food market on the local Warsaw market (local markets, small shops, shopping malls, discounters and supermarkets) were purchased. Information available to the consumer on the food (e.g. country of origin of the product, ingredients, information on allergens presence, nutritional value) was recorded.

The experimental material for the study consisted of samples of raisins and dried cranberries without packaging.

Microbiological analysis. The preparation of samples for microbiological analysis followed the procedure described in ISO 6887-1:2003. Samples were homogenized in a stomacher (Seward Stomacher 400 Circulator, UK) for 1 min at 300 units. Then the homogeneous samples were decimally diluted in Ringer's solution up to 10^{-4}. 1 mL of each prepared product solution was transferred into the Petri dish and mixed with 15 mL of plate count agar (PCA; BTL, Poland) and Sabouraud with chloramphenicol (BTL, Poland), in duplicate. The plates were incubated at 25 ±1°C for 72 h for yeast counting and for 120 h for mold counting according to ISO 21527-1:2008 and ISO 21527-2:2008, respectively. Molds were identified by genus on the basis of morphological features [Pitt and Hocking 2009].

Water content. The grounded samples (4–5g) were placed in a weighing glass and dried in a vacuum dryer (Memmert VO500, Poland) at 50°C under pressure of 1kPa for 24 h. The water content in fruit products was determined in triplicate.

Water activity (a_w) was measured using an AquaLab CX-2 (Decagon Devices Inc., USA) apparatus, in accordance with the manufacturer instruction. The temperature of water activity determination was constant (25°C). Each measurement was conducted in double repetitions.

pH determination – 30 cm^3 of distilled water was added to 10 g of the grounded material and the pH was measured using a Mettler Toledo pH meter. The measurement was performed in duplicate.

Statistical analysis was performed with the use of Excel 2016 and R Commander programs, using the Tukey's test at the significance level $p < 0,05$.

RESULTS AND DISCUSSION

Local market analysis of dried products sold without packaging

During the research period and at the research site, dry products sold in bulk were available in 63% of retail outlets. A total of 176 dried products (nuts were not included in the study) were found, including whole and cut (sliced and diced), dried or dried candied products, both from conventional and organic farming. It was noted that only 3 points of sale provided more complete information about the products, i.e. country of origin, product composition, information on allergens, manufacturer's name, date of minimum

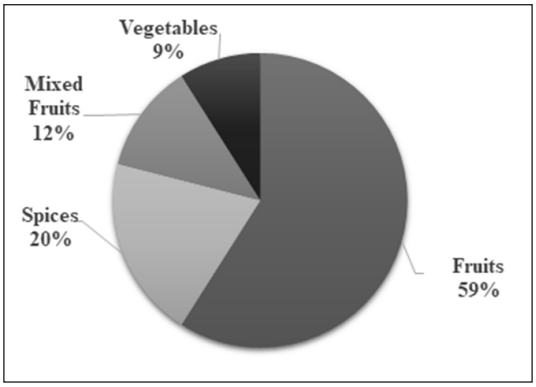


Fig. 1. Types of dried products sold without packaging available at the market during the study

Rys. 1. Rodzaje suszu sprzedawanego bez opakowania dostępne na rynku w trakcie badania

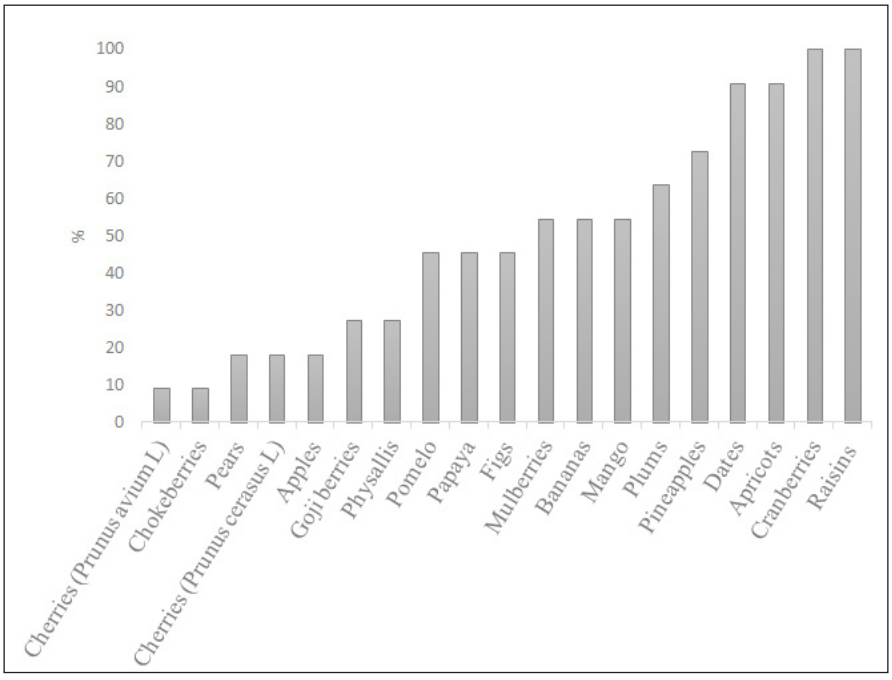


Fig. 2. Types of dried fruits sold without packaging

Rys. 2. Rodzaje suszonych owoców sprzedawanych bez opakowania

durability, recommended storage conditions, nutritional value of the product. Few places provided information on the nutritional value and composition of dried products. None of the visited shops had any information available on the fruit varieties used as raw material. The products selected to the analysis were exposed in various ways at points of sale, and the buyers could weigh or count the products without any restrictions. In most of the

large-format stores and neighborhood stores, the products were placed in closed vessels or plexiglass containers. Whereas at all marketplaces as well as 3 supermarkets, products were in open, uncovered boxes, plastic bags and containers.

The dominant group of products (Fig. 1) were dried fruits in separate form (59%) or available in the form of mixtures (12%). The share of vegetables was only 9%, spices accounted for 1/5 of the market for dried products sold in bulk.

Among the available fruits, the dominant group were raisins and cranberries (Fig. 2) followed by apricots, dates, pineapples and plums (available at over 50% of places). The lower number of places sold dried berry fruits (cherries, mulberries) and exotic or pome fruits (apples, pears). Dried fruits as chokeberries and cherries (*Prunus avium* L.) were offered only in one place. Raisins were available in different varieties, including those from organic farming (only 2 points of sale). Whereas cranberries were available as cut (44%) and whole, some after osmotic dehydration (78%). Producers' information available at the point of sale indicates that only 4 products had no sugar added. It was found that out of all examined raisins and cranberries, only two indicated the content of preservatives (sulfur dioxide), or information that the product may contain this substance. In the case of remaining samples, the sellers did not disclose the presence of chemical preservatives and other additives that could affect the shelf life of the final products.

Microbial and physicochemical characteristic of dried cranberries and raisins sold without packaging

The presence of fungi was detected in all the raisin samples available during the research period and at the research site. No yeast was detected in any of them. The content of molds in 1 g of raisins was from 1,9 $\log_{10}$ CFU to 2,75 $\log_{10}$ CFU. According to the methods of detection, there was no significant difference in the number of fungal colonies grown on Saboraoud and PCA media, both for raisins and cranberries (data not shown). The contamination by yeast and molds of cranberry samples ranged from absence in 0,1g (5 products) g to 10^2 CFU g^{-1}. The types and forms of the dried product did not affect the number of fungi contamination. Molds belonging to the genus *Aspergillus* sp. were dominant fungal microflora. *Rhizopus* spp. molds appeared only in two samples of raisins and *Cladosporium* spp. in one sample. The presence of them can be explained by their ubiquity and widespread in the environment. They can survive in a wide range of temperatures and environmental pH (especially *A. niger* withstands extremely low pH, approx. 1,50) and spores in the air can travel long distances [Krijgsheld et al. 2013]. No correlation was found between the number of fungi and the type of product, i.e. added sugar.

The presence of mold of the genus *Aspergillus* may be associated with contamination with mycotoxins such as aflatoxins, ochratoxin A, gliotoxins, fumomisins, sterigmatocystin or patulin [Azaiez et al. 2014]. The presence of molds of the genus *Penicillium* spp. in dried cranberries, *A. niger* and *Cladosporium* spp. in raisins was detected in by Tournas et al. [2015]. The presence of *Aspergillus* in raisins, apricots, dates (packed and without packaging), as well as *Rhizopus* in dates was found. Many of the mold in dates belonged to the genera *Aspergillus, Penicillium* and *Cladosporium* [Quaglia et al. 2020]. The presence of patulin has been shown in raisins (light and dark varieties) as well as in dried prunes, apricots or apples [Hartwig et al. 2019]. In the case of raisins, the pres-

ence of HT-2 toxin, ochratoxin A, eniatin B and aflatoxin G1 was detected [Azaiez et al. 2014]. Following the European Commission Regulation dried fruits must be analyzed for level of aflatoxins and ochratoxin. The maximum level of the sum of aflatoxins (B1, B2, G1, and G2) in raisins and other dried fruits is 4 ng/g, while the maximum ochratoxin A content for dried grape fruits (currants, raisins, and sultanas) is 10 ng/g [European Commision 2008]. 47% of raisin samples from Polish food market were contaminated with ochratoxin A and exceeded the maximum level 3.5 times [Hajok et al. 2019].

Some of the dried fruits examined in this work were stored in open packages or vessels, which made them exposed to contamination with the microflora present in the air, while some were covered or stored in various types of containers, which could reduce potential contamination to varying degrees. The time of exposure of the products to contamination, which may change as a result of product rotation, as well as the hygienic conditions in each store should also be taken into account. Product storage hygiene, i.e. the frequency of washing and disinfecting dishes and application equipment that come into contact with the product, as well as packaging used in stores, are undoubtedly important for the microbiological quality of unpackaged food.

Water activity (a_w) is an important factor that particularly influences the development of microflora. Food with a_w lower than 0.60 is considered to be microbiologically stable food. The mean water activity of cranberries and raisins did not differ significantly and amounted to 0.422 ±0.038 and 0.418 ±0.031, respectively. Raisins were characterized by a lower water content compared to cranberries, and at the same time a higher value of pH (Table 1). Average water content of raisins was 9.56 ±1.59%, while in the case of cranberries it was 11.11 ±2.10%. Comparable results for water content and activity were obtained by Pałacha and Mazur [2019], who determined the water content in cranberries at the level of 11.93%, and in raisins at the level of 11.99%. Low water content in the dried product proves that the drying process was successfully carried out.

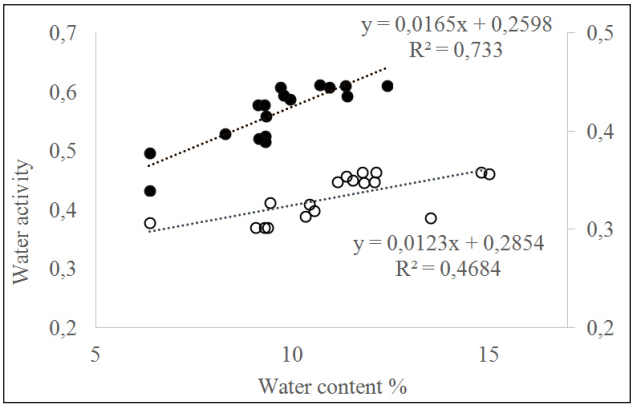
An attempt was made to correlate water activity and its contents in the examined dried products (Fig. 3). Value of the coefficient of determination $R^2 = 0.733$ confirms the better relationship between water activity and content water for raisins, compare to cranberries

Table 1. Minimum, maximum and average values of the measured physicochemical parameters of dried cranberries and raisins sold without packaging

Tabela 1. Minimalne, maksymalne i średnie wartości mierzonych parametrów fizyko-chemicznych suszonych owoców żurawiny i rodzynek sprzedawanych bez opakowania

Product Produkt	Value Wartość	Water content [%] Zawartość wody [%]	Water activity Aktywność wody	pH
Raisins Rodzynki	min.	6.35	0.340	2.64
	max.	12.39	0.448	4.04
	av.	9.56^{a} ±1.59	0.418^{a} ±0.031	3.70^{b} ±0.34
Cranberries Żurawina	min.	6.36	0.370	2.57
	max.	15.00	0.465	3.51
	av.	11.11^{a} ±2.10	0.422^{a} ±0.038	2.85^{a} ±0.23

The letters a, b stand for homogeneous groups in columns (Tukey's test at the significance level α = 0.05)\ Litery a, b oznaczają jednorodne grupy w kolumnach (test Tukeya na poziomie istotności α = 0,05)



*Black marks means cranberries (left OY axis) and empty marks goes for raisins (right OY axis)/Czarne znaczniki – żurawina (lewa oś OY), puste znaczniki – rodzynki (prawa oś OY)

Fig. 3. Water content (%) and water activity correlation of examined dried products

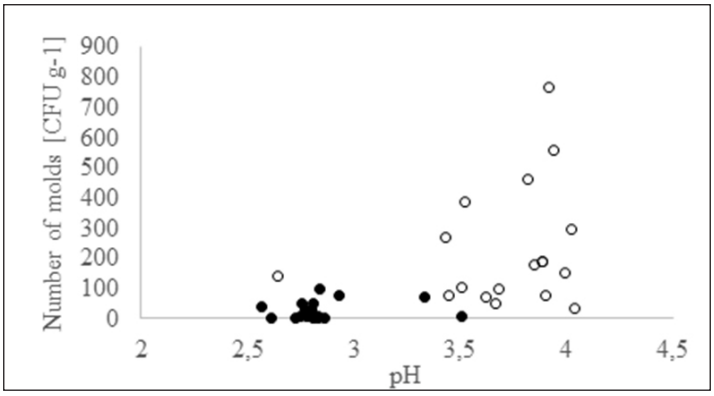
Rys. 3. Korelacja zawartości wody (%) i aktywności wody w badanych produktach suszonych

(R^2 = 0.4684). The significant difference in value of the coefficient these two types of samples, could be due to the difference in product composition. Dried fruit is a processed product, which may contain other ingredients that affect water activity in different ways. Water activity in food is strongly dependent on its composition, because as a complex multicomponent matrix, it can bind the water contained in it to varying degrees [Pałacha and Markiewicz 2011]. The points of sale most likely differed in air parameters, including relative humidity or temperature, which could have resulted in a partial change in water content at the time of sample purchase.

Another factor that significantly determines the microbiological stability of food is pH. The tested samples were characterized by low pH value (Table 1). The mean pH of the raisins was 3.70 ±0.34. The mean pH of cranberries was significantly lower and equaled 2.85 ±0.23. The dependence of the number of fungi on the pH in examined dried fruits was shown in Figure 4. Acid-rich fruits have a low pH what combined with various methods, speeds up the death of the microorganism. Pre-treating with an acidic solution (ascorbic, citric acid, etc.) enhances the destruction of potentially pathogenic bacteria during drying and storage, including *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*, and enhance the safety of dried fruits [DiPersio et al. 2003]. Therefore, the survival of microorganisms during drying can be related to the intrinsic factors of the food [Alp and Bulantekin 2021].

An attempt was made to determine the correlation between pH or a_w and the presence of fungi microflora (Table 2). In contrast to cranberries, the presence of fungi in raisins was more determined by the pH value than by low $a_{w,}$ although the correlation were low ($R^2 \leq 0.21$).

It should be emphasized that among the frequently used chemicals preserving dried products, sulphur dioxide, calcium sorbate and sodium metabisulphite are used. That compounds can inhibit the growth of mold in particular [Krzystofik et al. 2015]. Howev-



*Black marks means cranberries and empty marks goes for raisins/Czarne znaczniki – żurawina, puste znaczniki – rodzynki.

Fig. 4. The dependence of the number of fungi on the pH in examined dried fruits

Fig. 4. Zależność liczebności grzybów w badanych suszach od pH

Table 2. Number of molds, a_w and pH correlation of examined dried products

Tabela 2. Korelacja liczby pleśni, a_w i pH w badanych suszonych owocach

Product Produkt	Number of molds Ilość pleśni	
	a_w	pH
Raisins Rodzynki	$y = 0.0004\ x + 3.6283$	$y = 7E{-}05\ x + 0.4024$
	$R^2 = 0.0456$	$R^2 = 0.1890$
Cranberries Żurawina	$y = 0.0006\ x + 0.4066$	$y = 0.0013\ x + 2.8153$
	$R^2 = 0.2051$	$R^2 = 0.0315$

er, sulphur and sulphite compounds may lead to asthmatic reactions in some people. Antimicrobial properties of some fruit should be mentioned in the context of lower number of molds in cranberries. Compounds such as organic acids, anthocyanins, flavonoids, and phenolic compounds exhibit bactericidal as well as fungicidal properties [Stobnicka et al. 2013, Krzepiłko et al. 2020].

CONCLUSIONS

An attempt was made to correlate between water activity on water content of dried cranberries and raisins sold without packaging. The value of the coefficient of determination indicated a weak dependence a_w on water content of examined products. The examined products were characterized by proper physicochemical parameters, which resulted in low contamination with fungi microflora.

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ZANIECZYSZCZENIE GRZYBAMI SUSZONYCH OWOCÓW SPRZEDAWANYCH BEZ OPAKOWAŃ W KONTEKŚCIE AKTYWNOŚCI WODY, ZAWARTOŚCI WODY I PH

Streszczenie: Suszone owoce są sprzedawane w opakowaniach lub bez nich, a to implikuje różne czynniki zewnętrzne, które mogą wpływać na rozwój drobnoustrojów. Na jakość mikrobiologiczną produktów suszonych sprzedawanych luzem mogą mieć wpływ warunki higieniczne panujące w miejscu sprzedaży, czas i sposób ekspozycji oraz jakość powietrza. Suszona żywność jest higroskopijna zatem jej nieprawidłowe przechowywanie może skutkować zwiększeniem zawartości wody i tym samy stworzyć odpowiednie środowisko do rozwoju mikroroganizmów, w tym pleśni. Suszone owoce żurawiny i rodzynki sprzedawane bez opakowania charakteryzowały się odpowiednimi parametrami fizyko-chemicznymi, co skutkowało niskim zanieczyszczeniem mikroflorą pleśniową. Zaproponowano prosty

model matematyczny opisujący zależność aktywności wody i zawartości wody w badanych produktach. Wartość współczynnika determinacji wskazywała na słabą zależność aktywności wody od zawartości wody. Niższe obciążenie mikrobiologiczne próbek żurawiny korelowało z ich pH i naturalną obecnością związków o charakterze przeciwdrobnoustrojowym.

Słowa kluczowe: rodzynki, suszona żurawina, zanieczyszczenie pleśniami, aktywność wody, zawartość wody, pH