# MICROBIOLOGICAL STABILITY OF DRIED MARJORAM DURING STORAGE: THE ROLE OF WATER ACTIVITY AND PACKAGING

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## ABSTRACT

The aim of the study was to evaluate the microbiological stability of dried marjoram during a 6-month storage period under controlled conditions (20 °C, RH 50 ± 5%) and to determine the role of water activity and packaging type (paper vs. foil). Microbiological determinations were performed on the stored material for *Bacillus cereus*, *Clostridium* spp. and moulds. Due to the lack of possibility of reliable colony counting in a part of the samples, the results were analysed using a qualitative 0/1 system (presence/absence). The most frequently detected microorganisms were *Clostridium* spp. spores, whereas detections of *B. cereus* and moulds were sporadic and showed no time trend. During the same period, water activity and total water content were determined. The water activity of marjoram remained within the range typical for low-aw products (0.35–0.61), which mechanistically explains the observed microbiological stability. Under the tested conditions, no systematic influence of packaging type on the frequency of microorganism detection was found.

*Keywords: marjoram, water activity, packaging, Bacillus cereus, Clostridium spp., moulds*

## INTRODUCTION

Dried herbs and spices are natural products widely used in the food industry. Due to their low water activity, they are considered microbiologically stable; however, they may contain diverse microflora originating from soil and the cultivation environment (Witkowska et al., 2011; Fogele et al., 2018). Numerous studies have demonstrated the presence of high numbers of microorganisms in spices, including spore-forming bacteria such as *Bacillus cereus* and *Clostridium* spp., as well as moulds capable of producing mycotoxins (Fogele et al., 2018; McKee, 1995; García et al., 2001). Water activity (aw) is an important factor determining the possibility of microbial growth. Values of aw below 0.60 practically exclude the development of bacteria and significantly limit the growth of moulds (ISO 18787:2017; Beuchat et al., 2013). Under such conditions, observed detections of microorganisms most often reflect their survival rather than multiplication (Beuchat, 1981; Rahman, 2009). The type of packaging may influence product stability through different barrier properties against water vapour (Cicero et al., 2022; Robertson, 2016; Karam et al., 2021). However, under conditions of stable temperature and ambient humidity, this effect may be limited.

The aim of the study was to assess the microbiological stability of dried marjoram and to determine the role of water activity and packaging type in shaping the microbiological quality of the product.

## MATERIALS AND METHODS

### Research material and storage conditions

Dried marjoram originating from three producers was subjected to analysis. The product was stored for 6 months in two types of packaging: paper (P) and foil (F). Samples were analysed at 0, 2, 4 and 6 months. Storage was carried out in a climatic chamber under controlled conditions: temperature 20 ± 1 °C and relative air humidity 50 ± 5%.

### Sample preparation

From each batch, 1 g of material was weighed and homogenised in 9 ml of 0.1% peptone water, which constituted a $10^{-1}$ dilution.

### Microbiological determinations

Determinations for *Bacillus cereus* were performed in accordance with PN-EN ISO 21871:2007. Vegetative forms were plated from a $10^{-3}$ dilution on MYP agar (48 h, 30 °C). In parallel, CompactDry BC plates were used in accordance with the manufacturer's documentation (Shimadzu Diagnostics Europe, France). In order to detect spore forms, a heat-shock procedure was applied (80 °C, 10 min).

Detection and determination of *Clostridium* spp. were carried out in accordance with PN-ISO 15213:2005. Vegetative forms were plated from a $10^{-3}$ dilution on TSC agar with selective supplement, and incubation was conducted under anaerobic conditions at 37 °C for 48 hours. In order to detect spore forms, a heat-shock procedure was applied (80 °C, 10 min). Identification of isolated strains was performed using API rapid ID 32 A biochemical tests (bioMérieux, France), in accordance with the manufacturer's instructions, which enabled confirmation of the affiliation of selected isolates to the genus *Clostridium*.

Mould determinations were carried out in accordance with PN-ISO 21527-2:2009. Plating was performed using the surface method on PDA and SDA media, and incubation was carried out at 25 °C for 5 days. In the first sampling term, determinations on SDA medium were not performed for logistical reasons.

### Determination of total water content and water activity

Water content was determined in accordance with PN-91/R-87019. Water activity was measured in accordance with ISO 18787:2017 using a Rotronic HygroPalm 23-AW-A meter.

## RESULTS

### Water activity and total water content

The obtained aw values were within the range typical for low-water foods and showed no significant changes over time (Figure 1). Under conditions of RH 50 ± 5%, the product remained close to hygroscopic equilibrium, which limited the possibility of rehydration (ISO 18787:2017; Beuchat et al., 2013). In contrast, the analysis of total water content in marjoram revealed slight fluctuations between subsequent sampling terms; however, these changes were not systematic and did not show consistent differences between paper and foil packaging (Figure 2).

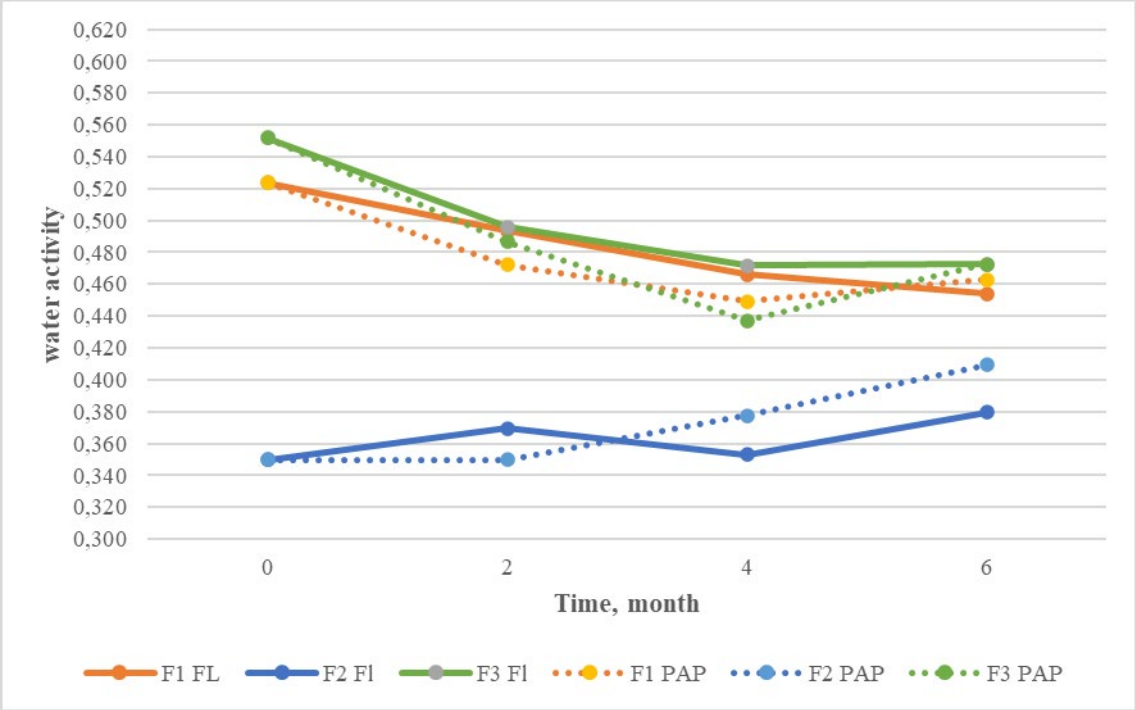


**Figure 1:** Water activity results during storage according to company and packaging type

*Notes: PAP – paper packaging; Fl – foil packaging; F1, F2, F3 - producers (companies) of dried marjoram*

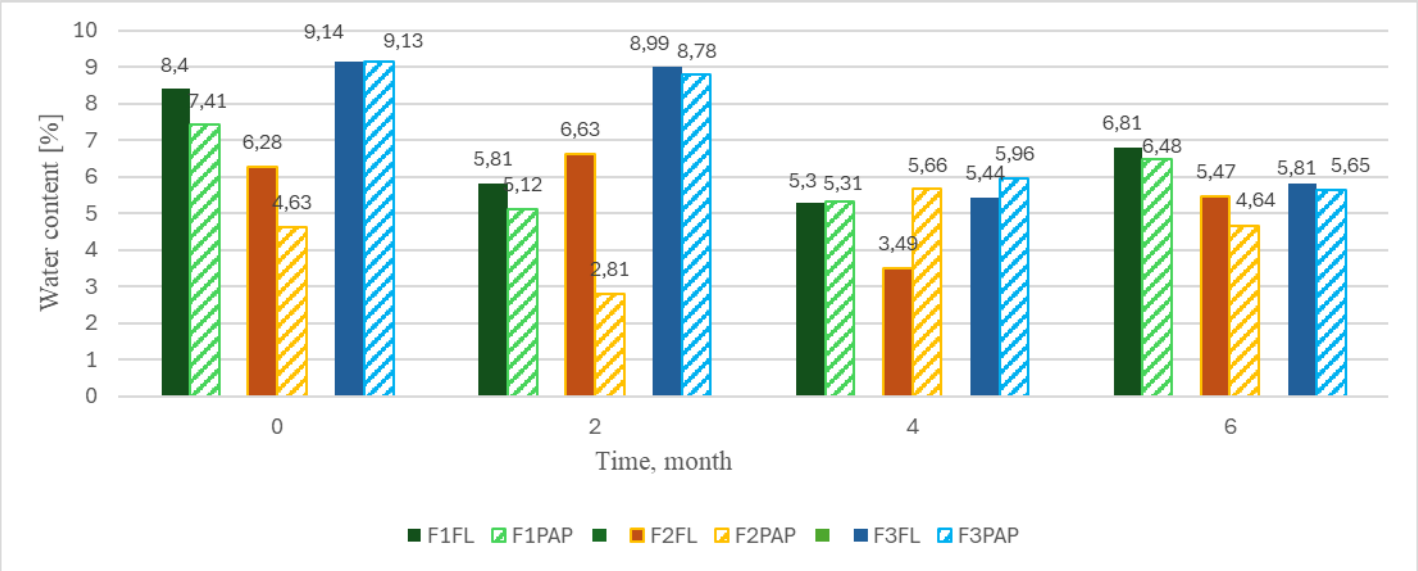


**Figure 2:** Total water content over time according to company and packaging type

*Notes: PAP – paper packaging; Fl – foil packaging; F1, F2, F3 - producers (companies) of dried marjoram*

**Microbiological profile**

The dominant group of microorganisms were *Clostridium* spp. spores, which is consistent with literature data concerning herbs and spices (Fogele et al., 2018; McKee, 1995; García et al., 2001; Schweiggert et al., 2007; Banerjee and Sarkar, 2003) (Tables 1 and 2). Vegetative forms of *Bacillus cereus* and moulds were detected only sporadically (Schweiggert et al., 2007; Banerjee and Sarkar, 2003) (Tables 1 and 2). The absence of a time trend confirms that the observed detections mainly reflected microbial survival rather than growth.

**Table 1:** Influence of packaging type on the frequency of microorganism detection in marjoram

| Parameter | Form | nP | %P | nF | %F | pp (p.p.) | RR | 95% CI (RR) | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| ***B. cereus*** | vegetative | 12 | 8.3 | 12 | 8.3 | 0.0 | 1.00 | 0.07–14.21 | 1.000 |
| ***B. cereus*** | spores | 12 | 25.0 | 12 | 25.0 | 0.0 | 1.00 | 0.25–4.00 | 1.000 |
| ***Clostridium* spp.** | vegetative | 12 | 41.7 | 12 | 33.3 | −8.3 | 0.80 | 0.28–2.27 | 1.000 |
| ***Clostridium* spp.** | spores | 12 | 75.0 | 12 | 66.7 | −8.3 | 0.89 | 0.53–1.49 | 1.000 |
| **Moulds (PDA)** | – | 12 | 8.3 | 12 | 25.0 | +16.7 | 3.00 | 0.36–24.92 | 0.590 |
| **Moulds (SDA)*** | – | 9 | 11.1 | 9 | 22.2 | +11.1 | 2.00 | 0.22–18.33 | 1.000 |

*Notes: nP – number of samples analysed in paper packaging; nF – number of samples analysed in foil packaging; %P – percentage of samples with detected microorganisms in paper packaging; %F – percentage of samples with detected microorganisms in foil packaging; pp (p.p.) – percentage point difference between packaging types (P vs. F); RR – relative risk (ratio of detection frequency in foil packaging to paper packaging); 95% CI (RR) – 95% confidence interval for relative risk; p-value – significance level from Fisher's exact test*

** For SDA n = 9/9 (no data available in the first sampling term)*

No statistically significant differences were found between paper and foil packaging in the frequency of detection of any of the analysed microorganisms ($p > 0.05$). The observed point differences were characterised by wide confidence intervals, which resulted from the small sample size and the binary nature of the assessment (Table 1).

**Table 2:** Differences between companies – frequency of microorganism detection in marjoram

| Parameter | Form | F1 % (n = 8) | F2 % (n = 8) | F3 % (n = 8) |
|---|---|---|---|---|
| ***B. cereus*** | vegetative | 0.0 | 0.0 | 25.0 |
| ***B. cereus*** | spores | 12.5 | 0.0 | 62.5 |
| ***Clostridium* spp.** | vegetative | 12.5 | 37.5 | 62.5 |
| ***Clostridium* spp.** | spores | 62.5 | 75.0 | 75.0 |
| **Moulds (PDA)** | – | 25.0 | 12.5 | 12.5 |
| **Moulds (SDA)*** | – | 16.7 | 16.7 | 16.7 |

*Notes: F1, F2, F3 – producers (companies) of dried marjoram; % – percentage of samples with detected microorganism; n – number of analysed samples per company*

** SDA: n = 6 per company*

The highest frequencies of detection of *B. cereus* (particularly spores) were observed in samples from company F3. For *Clostridium* spp., spores occurred with high frequency in all companies, which indicates the dominant nature of this group of microorganisms regardless of the producer (Table 2).

## DISCUSSION

The aim of the study was to evaluate the microbiological stability of dried marjoram during a 6-month storage period and to determine whether the type of packaging (paper vs. foil) affects the frequency of detection of selected microorganisms. Additionally, the role of water activity (aw) and total water content as potential factors influencing the microbiological quality of the product was analysed.

**Influence of water activity and water content**

The water activity values of marjoram obtained in this study were within the range characteristic for low-aw products (0.35–0.61) and showed no significant changes during storage (Figure 1). At the same time, total water content exhibited slight fluctuations between sampling terms and packaging types, which is a phenomenon typical for hygroscopic products stored under controlled relative humidity conditions (Figure 2).

It should be emphasised that water activity and water content are not equivalent parameters (Rahman, 2009). Water content indicates the amount of water present in the product, whereas aw determines its biological availability. In the analysed material, the observed changes in water content did not translate into significant changes in aw. This means that any differences in moisture between packaging types concerned mainly the bound water fraction, which does not affect the ability of microorganisms to grow. Stable, low aw values were therefore the main factor limiting microbial development during storage (Beuchat, 1981; Rahman, 2009).

The storage conditions applied in the experiment (20 °C, RH 50 ± 5%) favoured the maintenance of hygroscopic equilibrium of the product and minimised the risk of secondary rehydration. Under such conditions, the growth of vegetative bacteria and most moulds is practically impossible, which is reflected in the obtained microbiological results.

**Frequency of microorganism detection in marjoram**

The microorganisms most frequently detected in the analysed samples were *Clostridium* spp. spores. The high frequency of their presence (66.7–75.0%) is consistent with literature data indicating that spores of anaerobic bacteria are a typical component of plant raw material microflora and exhibit high resistance to environmental factors (Schweiggert et al., 2007; Banerjee and Sarkar, 2003). The presence of these forms should be interpreted as a remnant of the primary microflora rather than as a result of multiplication during storage.

Detections of vegetative forms of *Bacillus cereus* were sporadic in nature (8.3% of samples), which is consistent with low aw values preventing their growth. Similarly, detections of moulds on PDA and SDA media were infrequent and showed no increasing trend over time, confirming that the observed isolates mainly represented spore forms or spores already present in the initial product.

The absence of clear changes in the frequency of microorganism detection between successive sampling terms indicates the stable nature of marjoram microflora under storage conditions with low aw. These results confirm that in products with water activity below 0.60, the predominant phenomenon is microbial survival rather than multiplication.

**Influence of packaging type**

Comparison of microorganism detection frequencies between paper and foil packaging showed no statistically significant differences for any of the analysed parameters (Table 1). For all microorganisms, p-values obtained in Fisher's exact test exceeded the significance level of 0.05. Point differences (pp) between packaging types were small and were characterised by wide confidence intervals for the relative risk (RR).

The largest difference was observed in the case of moulds determined on PDA medium (pp = +16.7 p.p.; RR = 3.0); however, even in this case the result did not reach statistical significance, and the wide confidence interval indicates a substantial influence of the small sample size.

The obtained results suggest that under conditions of low and stable water activity, the type of packaging does not play a significant role in shaping the microbiological quality of dried marjoram. This means that potential differences in the barrier properties of packaging materials against water vapour were not sufficient to affect the aw level of the product to a biologically relevant extent.

**Study limitations**

An important limitation of the present study was the binary nature of microbiological assessment (0/1). The use of such an approach resulted from the lack of possibility of reliable colony counting in a part of the samples, which prevented quantitative analysis. Nevertheless, the qualitative method allows for a reliable assessment of microorganism presence and is acceptable in the case of products with very low microbial counts (International Commission on Microbiological Specifications for Foods (ICMSF), 2018).

An additional limitation was the lower number of mould determinations on SDA medium in the first sampling term (limitations independent of the research team). For this reason, analyses concerning SDA were based on a slightly smaller number of observations.

## CONCLUSIONS

Dried marjoram stored for 6 months under controlled conditions (20 °C, RH 50 ± 5%) was characterised by high microbiological stability, which resulted primarily from the low water activity of the product. Water activity values (aw) remained at a level below 0.60 throughout the entire storage period, effectively limiting the possibility of bacterial and mould growth. The microorganisms observed mainly represented spore forms present in the initial raw material. The most frequently detected microorganisms were *Clostridium* spp. spores, whereas detections of vegetative forms of *Bacillus cereus* and moulds were sporadic in nature and showed no increasing tendency over time. No statistically significant influence of packaging type (paper vs. foil) on the frequency of detection of *B. cereus*, *Clostridium* spp. or moulds was found. The differences obtained between packaging types were small, statistically non-significant and fell within wide confidence intervals. Changes in total water content observed between packaging types and storage terms did not translate into significant changes in water activity, which confirms that aw – rather than percentage water content – is the key parameter determining the microbiological stability of spices. The applied binary assessment method (0/1) enabled reliable analysis of microorganism presence in a product with a very low microbial load, although it limited the possibility of quantitative differentiation of contamination levels.

Under conditions of low and stable water activity, packaging type has no significant impact on the microbiological quality of dried marjoram. Both paper and foil packaging can be used for storage of this product without increasing microbiological risk, provided that appropriate storage conditions are maintained. An important element in ensuring the microbiological safety of spices is the maintenance of low water activity of the raw material and control of environmental conditions during storage and distribution (Beuchat, 1981; Rahman, 2009; Robertson, 2016). The obtained results indicate that technological and logistical measures should focus primarily on preventing secondary rehydration of products rather than on the selection of a specific type of packaging material.

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