Evolution of Microbial Flora during Storage of Minimally Processed Herbs

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Abstract

Post-harvest operations, handling and storage of fresh herbs may decrease the content of bioactive compounds and increase the microbial contaminants. Minimal processing can extend shelf-life and guarantee microbiological safety. The present study tested the efficacy of minimally processing of parsley (*Petroselinum crispum*), dill (*Anethum graveolens*) and lovage (*Levisticum officinale*) by monitoring the microbiota during cold storage. The three herbs were minimally processed and preserved at 4°C for 12 days. Total plate count and total yeast and mould count were determined during storage and correlated with sensory and biochemical parameters. Parsley had the highest microbial load and lovage the lowest total yeast and mould count throughout the study. Microflora increased significantly during the 12 days of storage. We found that microbial regulations lacked for minimally processed products; thus we could not compare the results with legal limits. Total plate count, total yeast and mould count were correlated negatively with the score for texture and content of total chlorophylls, vitamin C, and total polyphenols. The microbial load was also correlated with the content of volatile aroma compounds. In conclusion, the three minimally processed herbs had a high microbial contamination that increased with storage. So, in the absence of thermal processing, only legal limitations of microflora can guarantee shelf life and food security.

Keywords: dill, lovage, parsley, total plate count, total yeast and mould count

INTRODUCTION

Herbs contain bioactive compounds that promote health but they can be heavily contaminated with microorganisms. This microflora reduces shelf-life and may lead to food-related outbreaks (Campbell *et al.*, 2001; Elviss *et al.*, 2009; Naimi *et al.*, 2003; Pezzoli *et al.*, 2008). A recent WHO/FAO report stated that leafy vegetables – herbs included – represent a high microbiological risk for food safety because they are consumed fresh (Sessmentseries, 2008). Contamination comes from the environment (soil, water, insects), manure and unhygienic handling (Sharma *et al.*, 1984). Thus herbs contain a variety of aerobic and anaerobic bacteria, yeasts and moulds that proliferate during storage (Aziz *et al.*, 1998; Mandeel, 2005; Martins *et al.*, 2001; Mckee, 1995).

This is especially true for minimally processed herbs because processing damages the tissues making the product more susceptible to decay (Lopresti and Tomkins, 1997).

Many studies have identified the microorganisms present on herbs but few have considered the evolution of microflora during storage. Data are also scarce on the relation between contamination levels and the content of bioactive compounds.

We aimed to determine the evolution of microflora on herbs during storage and to correlate the results with the sensory and bio-chemical quality. We studied this correlation for three minimally processed herbs - parsley (*Petroselinum crispum*), dill (*Anethum graveolens*) and lovage (*Levisticum officinale*) during 12 days of storage at 4°C.

MATERIALS AND METHODS

Fresh parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), and lovage (*Levisticum officinale*) were purchased from a local market and minimally processed as previously described (Cătușescu *et al.*, 2012a, b). Samples were stored at 4°C and tested for microbial contamination in the 1st, 5th, 8th and 12th day of storage.

Total plate count (TPC) was determined using SR ISO 4833:2003 procedure: 5 g of sample were mixed with 45 ml of saline solution (0.85% w/v NaCl:H₂O) for 2 min (stomacher Minimix Interscience). Four decimal dilution were made; 1 ml of each dilution was poured and mixed in nutritive agar (Oxoid Nutrient Agar CM 0003). The plates were incubated head-down for 48 ± 2 h at 37°C. TPC was determined using equation 1.

$$TPC = \frac{\sum C}{M (n_1 + 0.1 \cdot n_2) \cdot d} \quad (1)$$

TPC – total plate count

∑C – sum of colonies per plate

Mv – mass of herb sample

n₁ – number of counted plates for the 1st dilution

n₂ – number of counted plates for the 2nd dilution

d – first dilution to be counted

Total yeast and mould count (TYMC) was determined using SR ISO 21527-1:2008 procedure: 0.1 ml of the dilutions prepared for TPC

were spread on DRBC agar (Oxoid DRBC Agar CM 0003). The plates were incubated for 5 days at 25°C. TYMC was determined using equation 2.

$$TYMC = \frac{\sum C}{M (n_1 + 0.1 \cdot n_2) \cdot d} \quad (2)$$

TYMC – total yeast and mould count

∑C – sum of colonies per plate

Mv – mass of herb sample

n₁ – number of counted plates for the 1st dilution

n₂ – number of counted plates for the 2nd dilution

d – first dilution to be counted

The sensory attributes, chlorophylls, total polyphenols, vitamin C and volatile aroma compounds were previously determined (Cătușescu *et al.*, 2012a, b).

Means, standard deviations, ANOVA (*p*-value < 0.05), Fisher (LSD, *p*-value < 0.05) and Pearson coefficient were computed using XLSTAT statistical software (MS Excel, Addinsoft, New York USA, Version 2012.4.03).

RESULTS AND DISCUSSION

Parsley was the most contaminated of the three herbs with a total plate (TPC) count of 3.9·10⁵ UFC/g (fig. 1). Some authors found a similar microbial load 5.78 log UFC/g for fresh-cut lettuce (Zhang *et al.*, 2006) but others reported higher values: 6 - 7 log UFC/g for lettuce, mint and cilantro (Trigo *et al.*, 2006).

Note: Different letters represent significant statistical differences Fisher ((LSD), *p* < 0.05).

Fig. 1. Total plate count (TPC) of minimally processed parsley, dill and lovage during storage

Note: Different letters represent significant statistical differences Fisher ((LSD), $p < 0.05$).

Fig. 2. Total yeast and mould count (TYMC) of minimally processed parsley, dill and lovage during storage

The TPC increased significantly for parsley until the 5th day of storage but remained constant for dill and lovage. The TPC of the two began to increase after this stage. At the end of trials the growth was of 30% for parsley, 22% for dill, and 25% for lovage. TPC was correlated strongly positively with storage ($r_{\text{parsley}} = 0.97$; $r_{\text{dill}} = 0.94$; $r_{\text{lovage}} = 0.96$; $p < 0.05$). Basbayraktar *et al.* (2006) and Lu *et al.* (2005) found increases below 1 log for minimally processed salad mixes stored at 5°C for 10 days and for minimally processed celery leaves stored at 4°C for 9 days. Trigo *et al.* (2006) determined much higher levels – up to 8 log UFC/g – for parsley stored at 4°C for 25 days.

Romania and Europe seem to lack regulations for the TPC and TYMC of minimally processed foods. Romanian standard – STAS 12550/87 – stated the quality of leafy vegetables and specified only sensory parameters with no microbial limits. The President of The National Authority for Sanitary and Veterinary Safety of Food (ANSVSA) gave the Order no. 27 in 6 June 2011 that specified microbiological restrictions for frozen vegetables but neglected fresh leafy vegetables (herbs included) and minimally processed products. The Regulation of European Commission (EC) No. 2073/2005 provides microbiological criteria for pre-cut fruit and vegetables (ready-to-eat) but limits only pathogens: *Salmonella* and *E. coli*.

Mohacsi-Farkas *et al.* (2006) suggest a target TPC of 10^6 UFC/g and a tolerated TPC of 10^7 UFC/g for the production day. A maximum TPC of 10^8 UFC/g indicated the end of shelf-life. We concluded that the three herbs had a microbial load below

the target TPC until the 12th day of storage which indicates a low initial contamination.

The TYMC of three herbs exceeded 10^3 UFC/g – the target proposed by Mohacsi-Farkas *et al.* (2006) – and approached the tolerated 10^4 UFC/g (Fig. 2). Parsley had the highest contamination. Lu *et al.* (2005) and Hammad *et al.* (2006) found similar values for celery leaves (3.54 log UFC/g), lettuce ($1.2 \cdot 10^4$ UFC/g), and salad mixes ($1.1 \cdot 10^4$ UFC/g). Basbayraktar *et al.* (2006) reported higher loads of yeasts and moulds for salad mixes (6.32 log UFC/g).

TYMC increased significantly during storage: by 0.58 log UFC/g in parsley, 0.57 log UFC/g in dill, and 0.69 log UFC/g in lovage. Parsley and dill had a similar load, while lovage had a significantly lower contamination level. TYMC was correlated strongly positively with storage ($r_{\text{parsley}} = 0.99$; $r_{\text{dill}} = 0.97$; $r_{\text{lovage}} = 1.00$; $p < 0.05$).

Similarly, Basbayraktar *et al.* (2006) observed an increase of 0.41 to 0.51 log UFC/g for salad mixes stored for 7 days at 5°C. Hammad *et al.* (2006) determined, however, increased TYMC: 1.14 log UFC/g for lettuce and 1.53 log UFC/g for green beans, both minimally processed and stored at refrigeration temperatures for 7 and 10 days, respectively. This suggested that TYMC increases dependent to the initial load and the product tested.

TYMC varied proportionally to TPC: $r_{\text{parsley}} = 0.98$, $r_{\text{dill}} = 0.98$, and $r_{\text{lovage}} = 0.88$ ($p < 0.05$).

Pearson correlation coefficient was used to determine the influence of TPC and TYMC on the sensory and bio-chemical quality of the three herbs.

The microbial load of dill and lovage was correlated negatively with texture (tab. 1). Their tissues softened when reaching the end of shelf-life - 14 days for dill and 15 days for lovage (Cătunescu *et al.*, 2012a). The decrease of firmness was caused by the physiological and microbial degradation of cellulose while the decrease of succulence was attributed to water loss through respiration.

Parsley showed no significant correlations between the microbiota and texture (tab. 1). It had the highest contamination level but the longest shelf-life, so its sensory quality changed least of the three herbs although.

The content of chlorophylls and vitamin C was correlated strongly with microbial load (tab. 2). These compounds oxidise with storage (Cătunescu *et al.*, 2012b) while contamination flora increases exponentially. Research shows, however, that chlorophylls have antimicrobial and antifungal activity that might explain this inverse relation (Kakkar, 2012; Maekawa *et al.*, 2007; Mowbray,

1957). Similar, vitamin C controls directly and indirectly the microbiota (Kallio *et al.*, 2012)

Several studies indicate polyphenols being responsible for the antimicrobial activity of plants (Tajkarimi *et al.*, 2010). In the present study, however, the content of total phenols did not correlate with microbial load (tab. 2). Thus, the content must be higher for polyphenols to control the contamination level.

The content and type of volatile aroma compounds affected the TPC and TYMC but the strength and direction of the correlation varied among the three herbs (tab. 3). The content of α -phellandrene was correlated strongly positively with the TYMC of dill but negatively for lovage. Its antibacterial activity was confirmed for plants from *Artemisia* and *Vetiveria* families (Duke, 2014). The content of β -myrcene and β -phellandrene was correlated negatively with the TYMC of dill. Both compounds have strong antifungal activity (Duke, 2014).

Tab. 1. Correlation of sensory texture with total plate count (TPC) and total yeast and mould count (TYMC) of minimally processed parsley, dill and lovage

Attribute	Pearson correlation coefficient (r)					
	parsley		dill		lovage	
	TPC	TYMC	TPC	TYMC	TPC	TYMC
Firmness	-0.19	-0.35	-0.99	-0.95	-0.97	-0.90
Succulence	-0.59	-0.70	-0.88	-1.00	-0.90	-0.97
Texture	-0.42	-0.56	-0.91	-1.00	-0.94	-0.99

Note: Values in bold are different from 0 with a significance level $\alpha=5\%$

Tab. 2. Correlation of chlorophylls, total phenols and vitamin C content with total plate count (TPC) and total yeast and mould count (TYMC) of minimally processed parsley, dill and lovage

Bioactive compounds	Pearson correlation coefficient (r)					
	parsley		dill		lovage	
	TPC	TYMC	TPC	TPC	TYMC	TPC
Chlorophyll a	-0.72	-0.63	-0.92	-0.66	-0.82	-0.63
Chlorophyll b	-0.86	-0.94	-1.00	-0.89	-0.60	-0.79
Total chlorophylls	-0.87	-0.82	-0.95	-0.73	-0.95	-0.83
Total phenols	-0.43	-0.40	-0.35	0.12	-0.60	-0.57
Vitamin C	-0.98	-0.99	-0.92	-0.98	-0.99	-0.97

Note: Values in bold are different from 0 with a significance level $\alpha=5\%$

Tab. 3. Correlation of volatile aroma compounds content with total plate count (TPC) and total yeast and mould count (TYMC) of minimally processed parsley, dill and lovage

Bioactive compounds	Pearson correlation coefficient (<i>r</i>)					
	parsley		dill		lovage	
	TPC	TYMC	TPC	TPC	TYMC	TPC
1.3.8-p-menthatriene	0.02	-0.07	-	-	-	-
2-methyl-1-propenyl-benzene	-0.24	-0.36	-	-	-	-
α-phellandrene	0.18	0.28	0.80	0.95	-0.80	-0.82
α-pinene	0.57	0.42	-0.82	-0.60	-0.44	-0.19
α-terpinolene	-	-	-	-	-0.70	-0.68
β- phellandrene	0.28	0.43	-0.81	-0.98	0.40	0.39
β-myrcene	-0.53	-0.50	-0.89	-0.99	0.92	0.80
β-pinene	0.17	-0.03	-0.88	-0.64	-0.41	-0.16
β-trans-ocymene	-	-	-0.62	-0.88	-	-
d-limonene	0.25	0.13	-0.74	-0.89	-0.91	-0.80
γ-terpinene	-0.78	-0.83	-	-	-	-
o-cymene	-0.49	-0.51	-0.47	-0.81	1.00	0.95
camphen	-	-	-	-	-0.35	-0.11
dehydro- <i>p</i> -cimene	-0.77	-0.82	-	-		
sabinene	-	-	-	-	-0.83	-0.65
terpinolene	0.11	-0.00	-0.95	-0.90	-	-

Note: Values in bold are different from 0 with a significance level $\alpha=5\%$

CONCLUSION

The three minimally processed herbs had a high initial contamination but below the TPC limit established by current research. This suggests the use of fresh herbs of high quality and good processing conditions. The herbs were heavily contaminated with yeasts and moulds and had a TYMC close to the tolerated limit of 10^4 UFC/g. Parsley was the most contaminated and lovage the least contaminated. The ranking was maintained until the 12th day of storage when the TPC and TYMC increased significantly for all three herbs.

The contamination microbiota was correlated with the texture, content of chlorophylls, vitamin C and volatile aroma compounds. The content of chlorophylls, vitamin C, β-myrcene, and β-phellandrene influenced negatively the TPC and TYMC of the three herbs.

We found a lack of European policy regarding the microbial criteria of minimally processed products. Only a strict limitation of TPC and TYMC

can guarantee the shelf-life and food security of the products in the absence of thermal processing.

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