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MICROBIOLOGICAL STATUS OF UNTREATED

HERBAL MATERIALS

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1. Introduction

The delicate flavour properties of plants used for herbal infusions require gentle processing to maintain their desirable qualities and this has a major influence on the other characteristics of the dried plant materials. All the key characteristics of herbal infusions are prime considerations for the European Herbal Infusion Association (EHIA) members in the provision of high quality products for domestic consumption.

One of the characteristics influenced in this way is the microbiological status of the herbs. There are a number of published surveys which review the microbiological quality of herbal infusion materials; those which are available have been carried out in Belgium (Devieeschouwer and Dony, 1979), France (Bernard, 1983), Germany (Frank, 1989; Kabelitz, 1996; Leombeck, 1987), Poland (Grabowska and Kedzia, 1982), the USA (Lerke and Faber, 1960) and Yugoslavia (Katusin-Razem et al 1988). These studies have shown that herbal infusion materials naturally have a higher level of micro-organisms than those found in most other foodstuffs. Surveys of culinary herbs and spices have observed similar levels of micro-organisms in dried plant material; these studies were carried out in Austria (Kneifel and Berger, 1994), Saudi Arabia (Grecz et al, 1986), South Africa (Baxter and Holzapel, 1982) and in the USA (McKee, 1995; Swab et al, 1982; Julseth and Deibel, 1974). In Germany (Schneider, 1987) the microbial status of pharmaceutical herbal plants from fresh materials to the dried products was investigated. As an example fresh Mint from the fields shows a TPC level between $5x10^5$ /g to 10^6 /g, after intermediate storing $2x10^6$ /g, after an air-separation process 10^6 /g and after the drying process between 10^4 and $4x10^5$ /g. Similarly, the level of Coliforms in the fresh material is between 10^2 and $4x10^5$ /g, after intermediate storing $2x10^4$ /g, after air-separating $2x10^5$ /g and after drying between 10^2 and $2x10^4$ /g.

The EHIA microbiology data base agrees with the published data; it comprises approximately 1000 samples analysed by member companies in Belgium, Germany, Great Britain, Italy, The Netherlands and Spain. The presence of such large numbers of micro-organisms is a natural consequence of the traditional agricultural practices and processing conditions in the countries where herbal materials are cultivated or harvested from wild plants. After harvest, the plant material is warm and moist which allows micro-organisms to multiply until the herbs have been sun-dried at ambient conditions.

Then the dried plant materials are carefully sorted and cleaned and finally packed for distribution so that consumers may prepare their preferred infusions with boiling water. The heat from the boiling water kills a large number of the micro-organisms present in the dry herbs thereby ensuring that the hot beverage is perfectly safe for consumption (Katusin-Razem et al, 1988; Leimbeck, 1987; Saint-Lebe et al 1985; Bernard, 1983).

Reduction of the number of micro-organisms in herbal materials is difficult as the use of post-processing treatments such as ethylene oxide for decontamination is banned in Europe (Directive EEC/89/365); the use of steam can cause a loss of volatile oil; and the use of irradiation, although an efficient means of reducing microbial contamination (Katusin et al, 1988; Lerke and Farber, 1960), is unacceptable to the consumer, although this attitude may be changing.

Recognising the need to understand levels of microbial contamination in their products, EHIA members have established a data base in order to monitor the microbiological status of herbal materials. In addition, the Microbiological Working Group has worked in recent years to establish guidelines for the microbiological status of products available for domestic consumption to ensure product safety. These guidelines reflect the quality of currently available materials from producing countries and can be used by Regulatory Authorities for their considerations.

The first step in establishing EHIA Microbiological Guidelines is to establish a current benchmark which is acceptable, however, it is even more important that producers should continue to improve their cultivation and processing practices. The use of the principles of Good Manufacturing Practice, Quality Assurance and Hazard Analysis of Critical Control Points can identify ways of improving hygiene and reduce microbiological contamination of the crops. EHIA has therefore used these principles as the basis of establishing a Good Agricultural Practice guideline document which was agreed in 1993 and is available to producers to assist them in developing improved agricultural methods.

2. European Limits of Microbiological Contamination of Foods

The presence of microbial organisms in food can be responsible for both spoilage and health hazards; it is therefore important to minimise such contamination of food and the consequences of microbial growth. Regulation of microbial contamination is difficult because of the dynamic nature of microbial populations; at normal ambient conditions in Europe (at least 15-20°C), if foodstuffs have a water activity above 0.65, a range of micro-organisms can double in number within 30 minutes. Control of microbial growth on foods using available preservation techniques and developing new ones is therefore a major concern of the food industry as a whole.

The infusion characteristics are preserved by drying the fresh plant material to capture the sensory qualities (aroma, flavour and appearance) and this also prevents microbial growth and spoilage. Herbal materials used for infusions as well as culinary herbs and spices are known to have naturally high levels of microbial organisms. The microbial contamination in herbal materials is from organisms found in soil, manure and airborne dust (Bernard, 1983). This is particularly relevant because the regions where these herbal infusion plants thrive are in non-industrialised countries with traditional cultivation and agricultural practices, often in hot and humid climates. Manual labour is used to harvest the crops and the plants are sun-dried in the open air. Under these conditions, the extent to which levels of micro-organisms can be controlled is subject to practical limits.

European regulation of microbial contamination in specific foods varies and is generally based on guidelines for the industry rather than by legislation. This is because microbial contamination of foodstuffs is dynamic in nature and the numbers of micro-organisms change throughout the lifetime of food depending on water activity, temperature and the methods of preservation used. A comparison of European Limits is shown in Table 1.

Table 1 European Microbial Control Limits

Microbial Contamination	Swiss Legislation[1]	GB PHLS Guidelines for ready to eat dried fruit & vegetables[2]			German Guidelines[3]				European Pharmacopoeia 1997[4]
	General Limits (convenience food)				Products which will be cooked; dried soups, sauces etc.		Spices		Herbal remedies prepared with boiling water
		Acceptable	Borderline acceptable	Not acceptable	Target	Max.	Target	Max.	
Total Plate Count	1 x 10^8/g	< 10^5	10^5 - < 10^6	≥ 10^6	1 x 10^7/g	-			1 x 10^7/g
E. coli	1 x 10^4/g	< 20	20 - < 10^2	10^2 - < 10^4	1 x 10^3/g	1 x 10^4/g	1 x 10^4/g		1 x 10^2/g
Salmonella	Absent in 25 g	Not detected in 25 g				Absent in 25 g		Absent in 25 g	
Yeast	1 x 10^7/g	-			-		-		
Mould and others	No visible contamination	-			1 x 10^4/g	1 x 10^5/g	1 x 10^5/g	1 x 10^6/g	1 x 10^5/g

1 Schweiz Verordnung über hygienisch-mikrobiologische Anforderungen, 1995

2 PHLS, 199?. Category 3 foods. These guidelines are more specific in their consideration of pathogens and include other limits because the foods are ready to eat and therefore not subject to any further heat treatment.

3 Bundesrepublik Deutschland. Mikrobiologische Richt- und Warnwerte zur Beurteilung von Lebensmitteln, 1988.

4 Ausgabe des Europaeischen Arzneibuches - Nachtrag 1998. Category 4 A limits.

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The control limits vary from 1 x 10^7/g - 1 x 10^8/g for Total Plate Count for foods, a level below 10^4 E. coli and require there to be no detectable Salmonella in 25 g. This level of microbial contamination is acceptable because the foods are subsequently going to be cooked or prepared with boiling water which will significantly reduce the number of viable micro-organisms to a small acceptable level and thus will not present any hazard after consumption. Guidelines in Great Britain (PHLS, 199?) for dried fruit and vegetables suggest a lower Total Plate Count of less than 10^6/g whilst requiring less than 10^2/g E. coli and that Salmonella be not detectable in 25 g because no further cooking is expected before consumption.

3. EHIA Microbiological Guideline

EHIA members agreed on a procedure for measuring microbiological contamination of herbal infusion materials in order to evaluate the current microbial status in commercially available materials. The objective was to identify a set of Microbiological Guidelines which will be the benchmark for what is acceptable and what is unacceptable within current practice. This paper therefore summarises the procedures, results and conclusions made from a statistical analysis of the data derived from approximately 1000 samples.

3.1 Experimental

Materials:

The investigations included Camomile, Linden, Mint, Orange Leaves, Verbena and other infusion materials to measure microbial populations.

Methods:

In general, EHIA prefers to use ISO methods to determine the microbiological level of contamination of herbal materials. The following methods were therefore used in these investigations.

Cut infusion material sampling plan:

Five random samples of 50 g are collected from the lot. The Salmonella test is carried out on 25 g samples from each of the five 50 g samples and can be pooled to a single 125 g sample. From the residue of two of the five samples 10 g is used for the remaining microbiological tests.

Uncut infusion material sampling plan:

Two random samples of 50 g and three random samples of 150 g are taken from the lot. The Salmonella test is carried out on 25 g samples from each of the two 50 g samples and each of the three 150 g samples and can be pooled to a single 125 g sample. From the three 150 g samples, three sub-samples of 100 g are put in a plastic bag and mixed well by shaking the bag. One sample of 10 g is used for the remaining microbiological tests.

Microbiology Tests:

Aerobic Plate Count: ISO 4833 Method entitled .Microbiology - General Guidance for enumeration of micro-organisms - Colony Count Technique at 30°C - 1991 was used.

Yeasts and Moulds: ISO 7954 Method entitled Microbiology - General Guidance for enumeration of yeasts and moulds - colony count techniques at 25°C - 1987 was used.

E. coli: The ISO 6391 Method entitled, Meat and meat products - enumeration of Escherichia coli - Colony Count Technique at 44°C using membranes - 1997 was used.

Salmonella: The ISO 6579 Method entitled, Microbiology - General Guidance Methods for the detection of Salmonella - 1993 was used.

3.2 Results and Discussion

Aerobic Plate Count / Total Plate Count

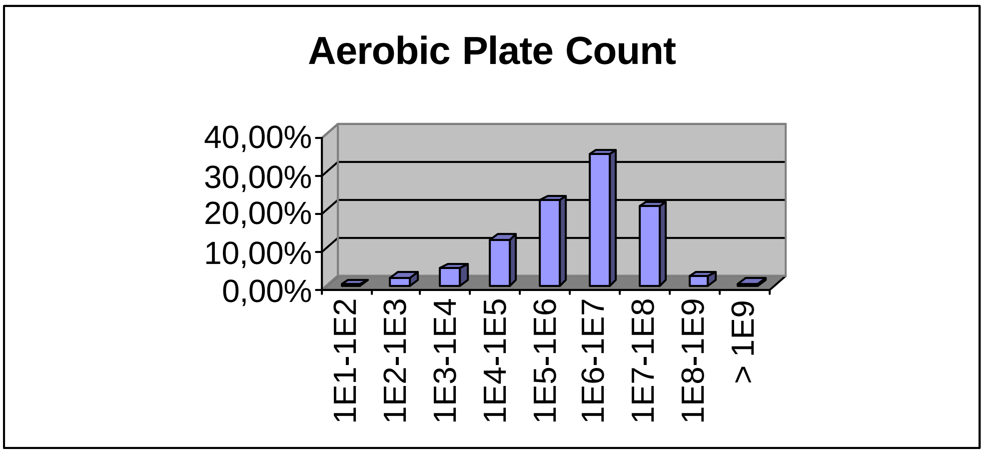
The major portion of the total plate counts ranges from 10^5 to 10^8 /g and is mostly concentrated between 10^6 and 10^7 /g, independant of the type of product.

- **Table 2.1 Aerobic Plate Count Range**

Product	Range (/ g)	Major Portion (/ g)
Camomile	$< 10^3 – 10^9$	$10^6 – 10^7$
Mint	$10^5 – 10^8$	$10^6 – 10^8$
Linden	$< 10^3 – 10^9$	$10^6 – 10^8$
Orange Leaves	$10^5 – 10^9$	$10^5 – 10^7$
Verbena	$< 10^3 – 10^8$	$10^5 – 10^8$

- **Table 2.2 Distribution of Aerobic Plate Count.**

Range	Percentage	Frequency
1E1-1E2	0,33%	3
1E2-1E3	2,21%	20
1E3-1E4	4,53%	41
1E4-1E5	12,15%	110
1E5-1E6	22,32%	202
1E6-1E7	34,48%	312
1E7-1E8	20,88%	189
1E8-1E9	2,43%	22
> 1E9	0,66%	6



Yeasts

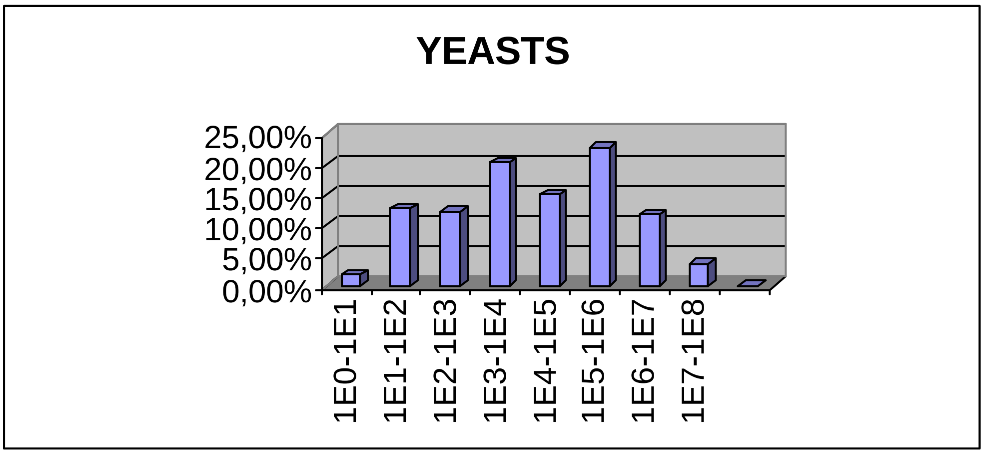
The major portion of yeasts ranges from 10^2 /g to 10^6 /g and is mostly concentrated between 10^4 /g and 10^6 /g, which is influenced by Mint. Excluding Mint the distribution is mostly concentrated between 10^3 /g and 10^5 /g.

- **Table 3.1 Yeasts Range**

Product	Range (/ g)	Major Portion (/ g)
Camomile	$< 10^1 – 10^6$	$10^3 – 10^4$
Mint	$10^1 – 10^8$	$10^4 – 10^7$
Linden	$< 10^1 – 10^6$	$10^1 – 10^4$
Orange Leaves	$10^1 – 10^4$	$10^1 – 10^4$
Verbena	$10^1 – 10^7$	$10^3 – 10^6$

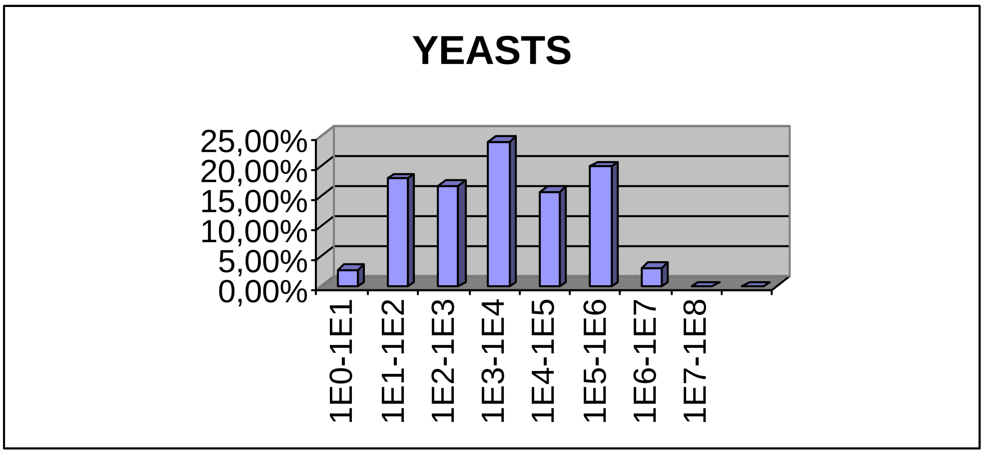
- **Table 3.2 Distribution of Yeasts, including Mint**

Range	Percentage	Frequency
1E0-1E1	1,90%	10
1E1-1E2	12,75%	67
1E2-1E3	12,19%	64
1E3-1E4	20,19%	106
1E4-1E5	15,05%	79
1E5-1E6	22,67%	119
1E6-1E7	11,62%	61
1E7-1E8	3,62%	19



▪ **Table 3.3**
Distribution of Yeasts, excluding Mint

Range	Percentage	Frequency
1E0-1E1	2,67%	10
1E1-1E2	17,91%	67
1E2-1E3	16,58%	62
1E3-1E4	24,06%	90
1E4-1E5	15,78%	59
1E5-1E6	19,79%	74
1E6-1E7	3,21%	12
1E7-1E8	0,00%	0



Moulds

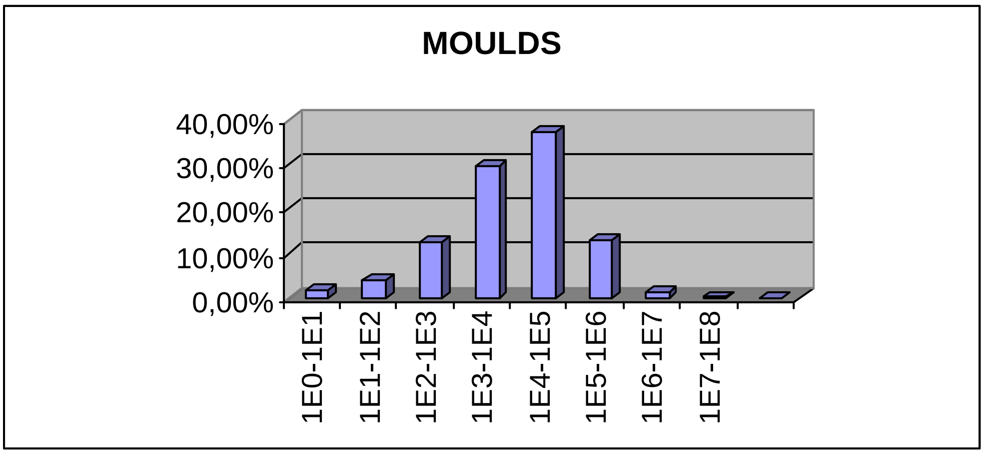
The major portion of moulds ranges from 10^3 /g to 10^6 /g and is mostly concentrated between 10^4 /g and 10^5 /g.

▪ **Table 4.1 Moulds Range**

Product	Range (/ g)	Major Portion (/ g)
Camomile	$< 10^1 – 10^6$	$10^1 – 10^5$
Mint	$10^2 – 10^8$	$10^3 – 10^5$
Linden	$10^2 – 10^5$	$10^2 – 10^5$
Orange Leaves	$< 10^1 – 10^5$	$10^3 – 10^5$
Verbena	$10^2 – 10^7$	$10^3 – 10^5$

▪ **Table 4.2 Distribution of Moulds**

Range	Percentage	Frequency
1E0-1E1	1,93%	13
1E1-1E2	4,16%	28
1E2-1E3	12,63%	85
1E3-1E4	29,42%	198
1E4-1E5	37,15%	250
1E5-1E6	12,93%	87
1E6-1E7	1,49%	10
1E7-1E8	0,30%	2



E.coli

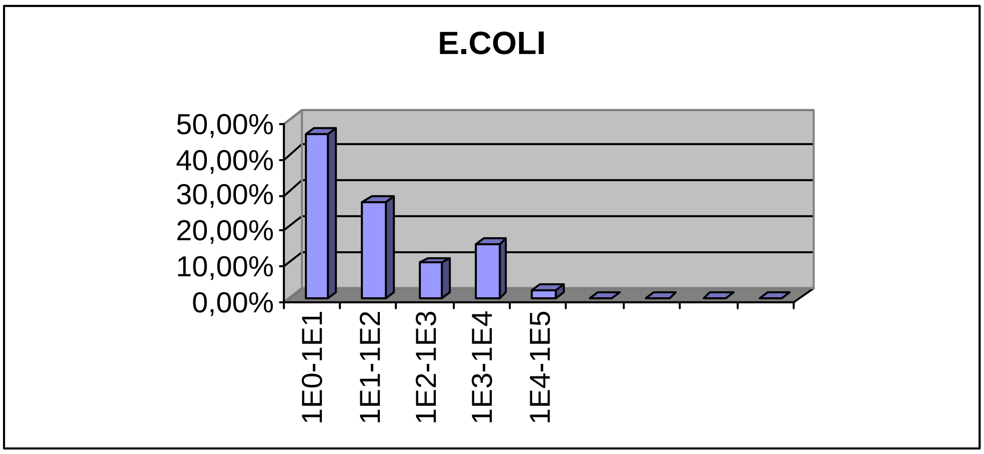
The major portion of E.coli ranges widely between 10^0 to 10^4 /g and is mostly concentrated between 10^0 and 10^2 /g.

▪ **Table 5.1 E.coli Range**

Product	Range (/ g)	Major Portion (/ g)
Camomile	$< 10^1 – 10^5$	$< 10^1 – 10^4$
Mint	$< 10^1 – 10^5$	$< 10^1 – 10^4$
Linden	$< 10^1 – 10^4$	$< 10^1 – 10^4$
Orange Leaves	$< 10^1 – 10^2$	$< 10^1 – 10^2$
Verbena	$< 10^1$	$< 10^1$

▪ **Table 5.2 Distribution of E.coli**

Range	Percentage	Frequency
1E0-1E1	45,71%	112
1E1-1E2	26,94%	66
1E2-1E3	9,80%	24
1E3-1E4	15,10%	37
1E4-1E5	2,45%	6



The summary of the investigations is presented in Table 6 as ranges of colony forming units (cfus) for Camomile, Mint, Linden, Orange Leaves, Verbena and Hibiscus.

Table 6 Micro-organism levels and water content of herbal infusion products measured by EHIA members

Product	**Aerobic Plate Count**		**Yeasts**		**Moulds**		**E. coli**		**Water Content**	
	Range (/g)	Major Portion (/g)	Range (/g)	Major Portion (/g)	Range (/g)	Major Portion (/g)	Range (/g)	Major Portion (/g)	Moisture (%)	Water Activity (Aw)
Camomile	< 10^3 - 10^9	10^6 - 10^7	< 10 - 10^6	10^3 - 10^4	< 10 - 10^6	10 - 10^5	< 10 - 10^5	< 10 - 10^4	6.5 - 11.9	0.35 - 0.66
Mint	10^5 – 10^8	10^6 - 10^8	10 - 10^8	10^4 - 10^7	10^2 - 10^8	10^3 - 10^5	< 10 - 10^5	< 10 - 10^4	8.2 - 14.0	0.28 - 0.65
Linden	< 10^3 - 10^9	10^6 - 10^8	< 10 – 10^6	10 - 10^4	10^2 - 10^5	10^2 - 10^5	< 10 - 10^4	< 10 - 10^4		
Orange Leaves	10^5 – 10^9	10^5 - 10^7	10 - 10^4	10 - 10^4	< 10 - 10^5	< 10 - 10^5	< 10 - 10^2	< 10 - 10^2		
Verbena	< 10^3 - 10^8	10^5 - 10^8	10 - 10^7	10^3 - 10^6	10^2 - 10^7	10^3 - 10^5	< 10	< 10		

The critical issue of water activity (Table 6) in the herbal materials was found to be below 0.66. This shows that herbal materials stored under normal conditions have water activities below the critical limits of 0.7 for mould growth and 0.85 for bacterial growth (Rockland & Beuchat, 1987).

A summary of the microbiology studies shows that the Total Plate Counts (TPCs) fall between 10^5 and 10^8/g, the majority being between 10^6 and 10^7/g. In the case of Yeasts, the range falls mainly between 10^3 and 10^6/g. Camomile, Linden and Orange Leaves range mainly between 10^1 and 10^4/g and Mint and Verbena fall mainly between 10^3 and 10^7/g. With Moulds the range is mainly between 10^3 and 10^5/g with the majority falling between 10^4 and 10^5/g. Finally with E.coli, there is a wide range between 10 and 10^4/g with the majority being below 10^2/g. The results for E.coli do not appear to be a normal distribution of data because the frequency peaks both between 10 and 10^2/g and between 10^3 and 10^4/g.

4. Comparison of the results with data from literature

These results are consistent with those of similar studies published in the scientific literature. A recent study with Mint observed the majority of the TPCs in the range 10^4 and 10^5/g; Yeasts and Moulds between 10^3 and 10^4/g; and for E.coli below 10^2 (Koszegi, 1995). Another study in 1989 found TPCs in various infusion materials between 10^4 and 10^7/g. Acid products with low pHs such as Hibiscus or Rose Hip had relatively low TPCs, as would be expected (Frank, 1989). The highest values were found in very light flowers or leaves such as Mint and Camomile; Yeast and Mould levels were found to be between 10^3 and 10^5/g. A study of Camomile showed the majority of TPCs were between 10^4 and 10^7/g; for Yeasts and Moulds between 10 and 10^5/g; and for E.coli between 10^2 and 10^4/g (Kabelitz, 1996). Yde et al. (1981) also showed that TPCs for herbal infusions ranged between 10^5 and 10^7/g, E.coli was detected largely below 10^2/g and Yeasts and Moulds were found between 10^4 and 10^5/g. Thonke et al., 1991 showed in therapeutic preparations from plant material had TPC levels of between 10^7 and 10^8/g and for Mould between 10^5 and 10^6/g. These authors summarised their results with those in other similar published studies and proposed that when herbal preparations are used for extracting active principles it is acceptable to have up to 5 x 10^8/g TPCs and up to 10^8 Mould as levels of contamination in starting materials so long as microbially induced changes in the plants were not visible. Finally, another study by Leimbeck in 1987 showed that Mint and Camomile had more than 80% of the TPCs in the range above 10^6/g and the majority of Yeasts and Moulds between 10^2 and greater than 10^4/g for Camomile and Mint.

These herbal materials are cultivated and harvested to manufacure products which will be infused to prepare hot beverages. It is the hot beverage which is consumed and it is necessary to understand the reduction of microbial numbers after infusion with boiling water; this is the equivalent to the role of cooking with culinary herbs and spices. A number of studies have shown that preparing infusions with boiling water results in a significant reduction in microbial contamination (Katusin-Razem et al, 1988; Leimbeck, 1987; Saint-Lebe et al, 1985; Bernard, 1983; Yde et al, 1981). This reduction in numbers of micro-organisms minimises any hazard which might be associated with consumption of these beverages. These studies have also shown that preparation of infusions with water at the sub-optimal temperature of 80°C is still sufficient to ensure safety for human consumption. Nevertheless, it is recommended that consumers infuse herbal drinks with boiling water for the best results.

5. Conclusion

The results of investigations published in the scientific literature show that microbial contamination of herbal infusion materials and culinary herbs and spices is similar and naturally higher than other foodstuffs. The results measured by EHIA members are consistent with these published results. The levels of micro-organisms can be related directly to the traditional agricultural practices in the countries of origin together with the mild conditions used in processing the harvested crops. These mild conditions help to preserve the desirable herbal aroma, flavour and colour characteristics of the hot beverages. Having considered all the issues, EHIA members have therefore established a Microbiological Guideline (Table 7) which ensures the safety of herbal materials infused with boiling water.

Table 7 EHIA Microbiological Guideline

Microbial Contaminant	Limit
Aerobic Plate Count	1.0×10^8/g
Yeasts[1]	1.0×10^6/g
Moulds	1.0×10^6/g
E. coli	1.0×10^4/g
Salmonella	Absent in 5 x 25 g
1 For Mint no Yeasts specification applies because of the high natural yeast load	

Summarising, EHIA has reviewed the issues relating to the status of microbial contamination of herbal infusion materials and identified that the products supplied for domestic consumption have levels which are consistent with those reported in the scientific literature. The levels are similar to other dried plant materials such as culinary herbs and spices. Both are subject to further preparation either with boiling water or by cooking at high temperatures which significantly reduces the numbers of micro-organisms present in the foodstuff which is consumed. This has resulted in EHIA publishing an agreed Microbiological Guideline (Table 7) to ensure that products distributed for domestic consumption are safe. Adherence to these guidelines is being achieved through implementation of Quality Assurance (QA) systems to check raw materials and verified using appropriate laboratory analysis.

EHIA member companies are also promoting knowledge transfer to the countries of origin in order to minimise the risks of food-borne disease at source. The Good Agricultural Practice document has been issued and its members are promoting this together with the principles of QA and Hazard Analysis of Critical Control Points to identify and implement further improvements which can be made to production techniques. In practice this is being accomplished by visiting suppliers in producing countries and through discussions and audits of their agricultural practices.

Nevertheless, EHIA member companies clearly label their products to recommend that consumers use boiling water to prepare infusions thus ensuring the best results for both taste and safety of the beverage consumed.

6. Conclusions

1 Dry herbal infusion materials are natural products which are cultivated, harvested and processed by natural, traditional processes.

2 Dry plant material processed by natural methods, including those used for herbal infusions, naturally have a higher lever of micro-organisms than those found in most other foodstuffs.

3 The levels of water activity in herbal infusion materials stored under normal conditions are sufficiently low to safeguard against the growth of micro-organisms.

4 Herbal infusion materials which comply the EHIA Microbiological Guideline (Table 7) are safe for consumption when prepared with boiling water, which effectively removes any remaining risk.

5 Chemical decontamination techniques (such as ethylene oxide treatment) to reduce the level of micro-organisms in dried plant material are banned by the EU, nor do EHIA members wish to use them.

6 Further quality improvements are desirable and are being promoted by EHIA member companies to ensure the availability of the best quality herbal infusions. This includes promoting the application of such generally accepted practices as GAP, GMP, QA systems and HACCP in the countries of origin.

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