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A NEW METHOD FOR IMPROVING THE QUALITY OF PEROXIDISED OILS

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SUMMARY

Soybean, sunflower, olive and black cumin oils were peroxidised by heating at 100 °C in the presence of atmospheric oxygen until the peroxide value was in the range of 121 - 135 meq./Kg oil. A proposed method was used to decrease the peroxide content of the peroxidised oils by shaking them with an acidic solution of freshly prepared ferrous sulfate. Some physico-chemical constants were determined on non-peroxidised, peroxidised and treated-peroxidised oils to illustrate the effectiveness of ferrous sulfate method towards increasing the keeping quality of the oils. The results demonstrate that shaking the peroxidised oils with the acidic ferrous sulfate solution caused remarkable decrease in the levels of peroxides and secondary oxidation products and also completely removed the polymers.

KEYWORDS: Peroxidised oils, oil quality assurance tests, oil quality improvement, ferrous sulfate method

INTRODUCTION

Lipids are considered as an important component of the diet being the principle source of energy, essential fatty acids and fat-soluble vitamins. However, lipids from various sources deteriorate at different rates and produce distinct off-flavour [1]. Most of the flavour compounds in lipids are produced by the reaction of atmospheric oxygen with the unsaturated fatty acids in triglycerides [2]. The development of objectionable flavour compounds by oxidation has significant damaging effects on consumer acceptability of edible oils. In this respect, there is evidence that in the body lipid oxidation may cause inflammation and play a role in some diseases, such as arthritis. atherosclerosis, heart disease or breast and colon cancer. In addition, free radicals of polyunsaturated fatty acids can be formed in living systems and oxygen can react with the radicals to form peroxy radicals and hydroperoxides. These compounds can chain-react to cause membrane damages [3].

Vegetable oil consumption has been increasing and currently surpasses animal fat consumption. This trend represents a change in the fatty acid composition of the diet. With the change in diet consumption over the past 50 years, dietary linoleic acid utillzation has increased the most and the saturated fatty acid consumption has decreased [4]. It is well-known that lipid oxidation depends on the number of double bonds in their moieties. Therefore, the change in human consumption and relying on diet containing high levels of linoleic acids leads to find ways to increase the diets shelf-life.

Many efforts have been made to prevent or delay deteriorative reactions in foods. In order to retard the undesirable changes in lipids due to oxidation it is necessary to add antioxidants to lipids before use in food products [5, 6]. BHA and BHT, the most widely used synthetic antioxidants, possess remarkable efficacy in various food systems to prevent their deterioration. Toxicological studies with BHA have been performed with rats, rabbits, swine and primate animal models. Time and dose-dependent changes in forestomach epithelium include hyperplasia, papillomas and carinomas [7-9]. BHT produced positive chromosomal aberrations in anaphases of human IW-38 embryonic lung cells [10]. Both BHA and BHT can promote bladder carcinogenesis in rats [11]. These findings demonstrate that their use in food has been questioned due to their suspected action as promotors of carcinogenesis as well as being due to a general rejection of synthetic food additives [12, 13]. Accordingly, it is highly desirable to remove or to lower the level of hydroperoxides in oils and food rich in oils without relying on the addition of synthetic antioxidants. The main goal of this work was to use a simple and cheap method to breakdown the hydroperoxides of some edible oils (sunflower oil, soybean oil, olive oil and black cumin oil). In addition, some physico-chemical constants were determined to compare the effectiveness of the proposed method using non-peroxidised, peroxidised and treated-peroxidised oils.

MATERIALS AND METHODS

Sources of oils and seeds

Sunflower (Hy-sun variety and black cumin seeds (local variety) were obtained from Oi1 Crop Department, Agriculture Research Center, Giza and Medicinal Plant Research and Propagation Station, Ministry of Agriculture, EI-Kanater EI-Kharyla, Egypt, respectively. Olive fruits (Picual variety) and soybean oil were obtained from Horticulture Research Institute and Soybean Factory, Food Technology Institute, Agriculture Research Center, Giza, respectively.

Solvents

All solvents used throughout the whole work were of analytical grade and distilled before use.

Oil extraction

Sunflower seeds, black cumin seeds and olive fruits were crushed and pressed by a laboratory hydraulic press. The extracted oils were dried over anhydrous sodium sulfate, filtered through Whatman filter paper No. 1 and kept in brown bottles at 5 °C until analysis.

Physico-chemical properties of oils

Refractive index, colour, acid value, peroxide value, iodine value and saponification number were determined according to A.O.A.C methods [14]. The degree of polymerization was determined for non-oxidised, peroxidised and treated-peroxidised oils according to the method of Peled *et.* al. [15].

Determination of iron (Fe) content

The method of Puchyr and Shapiro [16] was used for the determination of Fe in the oil samples (non-peroxidised, peroxidised and treated-peroxidised samples). This method consists of char-ashing and digestion followed by analysis via atomic absorption spectrophotometry (AAS Perkin Elmer 3300).

Peroxidation of oils

Peroxidation of oils was conducted by heating each oil (100 g) placed in a beaker (500 ml) at 100 °C for 10 days using a hot air dryer.

Removal of peroxides

The peroxidised oils (50 g) were shaken with freshly prepared ferrous sulfate solution (10 ml, 1 0%, w/v) in the presence of one drop of sulfuric acid (1N). Sodium chloride (10%, w/v) was added to break down the emulsion, then the oil was separated, dried over anhydrous sodium sulfate and filtered.

Designation of induction period with a rancimat

Rancimat 679 (Metrohm Ltd., CH-9100 Herisau, Switzerland) was used for the determination of oxidative stabilities of non-peroxidised, peroxidised and treated-peroxidised oils (sunflower, olive, soybean and black cumin oils). The induction time was automatically determined, i.e., the time from the start of the experiment to the intersection point [17].

Statistical analysis

The values of some physical and chemical constants and the induction periods of the oils were obtained in triplicate and the mean values are presented in Table 1. Analysis of variance of the two factor factorial design was applied for all data under the present study according to the method outlined by Snedecor and Cochran [18]. The L.S.D. test was used to compare the significant differences between means of treatments (Waller and Duncan [19]).

RESULTS AND DISCUSSION

Physico-chemical properties of some vegetable oils

Table 1 shows the physical and chemical properties of sunflower, black cumin, olive and soybean oils. Sunflower and soybean oils had comparable colour appearance. In general, the brightness of the oils was in the following decreasing order: black cumin>olive oil > soybean oil> sunflower oil. The colour values for the non-peroxidised, peroxidised and treated-peroxidised sunflower, black cumin, olive and soybean oils demonstrated that peroxidation and treatment process did not alter the colour appearance of these oils. The refractive indices indicated that soybean, sunflower and olive oils had approximately the same degree of unsaturation.

The acid and peroxide values for the oils under study were within the recommended values except for black oil which had higher values. According to the iodine values, black cumin and olive oils belong to drying oils, while soybean and sunflower oils belong to semi-drying oils. The saponification number of the oils demonstrates that these oils contained fatty acids with nearly the same chain-length. Table 1 indicates that the acid values of the peroxidised and peroxidised-treated oils were nearly the same. This means that the free acids (characterised by long-chain lengths) cannot be extracted with an acidic watery solution. Hence, the content of free acids was not altered in the oils treated with Fe^{2+} sulfate solution.

The peroxide value is one of the most commonly used method for measuring the hydroperoxides formed as the primary oxidation products in the oils. The peroxide values of the non-peroxidised, peroxidised and treated-peroxidised oils under investigation are shown in Table 1. It could be noticed that the peroxide value range of non-peroxidised oils was 0.95-11.11. After peroxidation of the oils the peroxide value range was 121.5 → 135.3 depending on the oil type. Upon treatment with freshly prepared acidic ferrous sulfate solution the peroxide value range for

TABLE 1 - Some physico-chemical properties of some vegetable oils.

Parameter	Sunflower oil			Soybean oil			Olive oil			Black cumin oil			LSD value at P = 0.05
	Non-Peroxidised	Per-oxidised	Treated-Peroxidised	Non-Peroxidised	Per-oxidised	Treated-Peroxidised	Non-Peroxidised	Per-oxidised	Treated-Peroxidised	Non-Peroxidised	Per-oxidised	Treated-Peroxidised	
Colour *Yellow* *Red* *Blue*	35 2.4 -	35 2.5 -	35 2.5 -	35 2.6 -	35 2.7 -	35 2.6 -	35 3.5 3.0	35 3.4 2.9	35 3.4 3.0	35 7.0 6.0	35 7.0 6.0	35 7.0 6.0	-
Refractive index at 25 °C	1.4724	1.4724	1.4724	1.4752	1.452	1.4752	1.4652	1.4652	1.4652	1.4554	1.4554	1.4554	-
Acid value	0.09a	3.00b	2.95b	0.95a	4.51b	4.53b	0.30a	3.95b	3.92b	5.50a	8.70b	9.60b	2.00
Saponificatio n number	191.00	191.00	191.00a	197.00	197.00	197.00	193.00	193.00	193.00	186.00	186.00	186.00	-
Iodine value (Hanus method)	128.00a	130.00a	129.50a	130.00a	133.00a	131.00a	83.00a	88.00a	85.00a	81.00a	84.00a	85.00a	5.00
Peroxide value (meq./Kg oil)	0.95a	121.50b	4.50a	1.75a	125.30b	4.30a	3.50a	130.50b	4.42a	11.11a	135.30b	5.18c	5.20
Induction period (hrs)	7.82a	1.5b	5.3c	8.3	1.00b	4.5c	23.00a	4.00b	10.50c	11.11a	1.50b	6.00c	0.0
TBA value	0.011a	0.985b	0.259c	0.087a	0.958b	0.204c	0.099a	0.987b	0.233c	0.159a	0.996b	0.298c	0.20
Polymer content (%)	0.0a	5.8b	0.0a	0.0a	4.3b	0.0a	0.0a	3.9b	0.0a	0.0a	4.5b	0.0a	1.00
Iron content (mg/ kg oil)	0.07	0.07	0.07	0.07	0.07	0.07	0.05	0.05	0.05	0.07	0.07	0.07	-

Numbers in the row followed by the same letter are not significant different at P = 0.05 relative to non-oxidised oil for each parameter.
TBA value = 2-thiobarbituric acid value

the oils was 4.3-5.18 rneq/kg oil. These results demonstrate that the peroxide values of the peroxidised oils were about 26 times as high as in the treated oils. In other words, the treatment with ferrous sulfate enormously reduced the peroxide values of the oils and they are within the acceptable range for human consumption. It is worth-mentioning that the temperature used for oil peroxidation (100 °C) was extremely lower than the usual temperatures for frying (150-180 °C). In fact, the main objective of this work was to obtain oils characterised by high peroxide content and to examine the efficiency of Fe^{2+} sulfate solutions in the elimination of peroxides. Therefore, the plan of the present study was the characterization of a model system and the future work would be carried out with frying oils (oils removed from frying equipment). The degree of polymerization for non-peroxidised oils was nill, whilst, the range of polymerized compounds in the peroxidised oils was 3.9-5.8%. The treatment with acidic ferrous sulfate solution completely removed the polymers from the oils under study (Table 1).

The TBA levels of the non-peroxidised oils were low (0.01-0.16) and followed the decreasing order: black cumin > olive > soybean > sunflower oil. The contents of the secondary oxidation products of the peroxidised oils were quite higher (0.96-1.00) and these peroxidised oils had the same TBA values. On the other hand, the TBA levels of the peroxidised oils were nearly 3-4 times as high as the non-peroxidised oils. It is noteworthy that the TBA values of the treated oils lay between 0.20-0.30 and the treated black cumin had the highest TBA value. Therefore, the proposed treatment remarkably reduced the TBA values of the peroxiclised oils. One would-point out that the concentration of aldehydes is reduced by the Fe^{2+} sulfate solution, but it was significantly higher than in non-peroxidised oils. Nevertheless, the treated oils did not induce any unpleasant odour.

It has been reported that iron increased lipid oxidation [20]. Accordingly, one would expect that treating peroxidised oils with freshly prepared acidic ferrous sulfate solution might induce further increase in oil oxidation rather than breakdown of the peroxides formed. Therefore, the induction periods of non-peroxidised, peroxidised and treated-peroxidised oils were determined as well as the levels of Fe in the oils. The data in Table 1 show that Fe content was nearly the same (0.05-0.07 ppm) in all oil samples. The induction periods for the non-peroxidised oils were remarkably higher than those the peroxidised oils, being about 4.3; 8.3; 5.8 and 7.4 times as high as that of the peroxidised sunflower, soybean, olive and black cumin oils, respectively. On the other hand, the induction periods of the non-peroxidised oils were approximately 1.5, 1.8, 2.2 and 1.9 as high as those of the treated sunflower, soybean, olive and black cumin oils, respectively. These findings lend weight on the effectiveness of the proposed method for the breakdown of the peroxides in oils. Consequently, one would suggest to apply the proposed method to lower the peroxide and polymer contents of fried oils.

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QUALITY STABILISATION OF FRESH HERBS USING A COMBINED VACUUM-MICROWAVE DRYING PROCESS

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SUMMARY

The quality of dried herbs mainly depends on three parameters: colour, aroma, and absence of off-flavour defects. The quality evaluation of dried products should always be related to the properties of the original fresh product. Especially convective hot-air drying (HAD) causes heat damage of the plant tissue and therefore affects the quality of the final product. The present study involves the investigation of vacuum-microwave drying (VMD) as a processing method for drying herbs. Colour, aroma, and off-flavour of vacuum-microwave dried parsley was evaluated and compared with corresponding properties of hot-air dried parsley (at 75 °C). VMD-parsley was greener in colour, exhibited a higher content of essential oils and less hay-like off-flavour defects than those prepared by HAD. In detail, L*a*b*-colour measurement revealed that VMD-samples had higher green hues directly after drying than those prepared by HAD. Also, the colour retention over 8 weeks, when stored in darkness, is significantly higher by using a vacuum-microwave drying process in comparison with convective drying. Colour degradation was observed in all samples, but was faster for hot-air dried parsley. Therefore a visible colour improvement of dried herbs can be obtained, but the coherence of final water content with colour stability must be considered. As can be seen, the rate of chlorophyll degradation increases with an increase of water content in the final product. Investigation of the impact of drying methods on the flavour quality showed, that VMD preserved over 90 % of the essential oils during processing compared with only 30 % by HAD. Furthermore VMD-samples were rated better than HAD-samples by a sensory panel for parsley-like, green-grassy and hay-/straw-like odour impressions as well as the general impression related to fresh parsley.

KEYWORDS: herbs, drying, colour, aroma, off-flavour, vacuum - microwave, parsley

INTRODUCTION

Herbs are the leafy parts of annual or perennial shrubs or plants and are distinguished by a high content of aromatic substances. They are used to flavour foods or beverages from ancient times. There is a general preference for using them fresh, but fresh cut herbs are seasonal and perishable commodities. Their limited availability and usability lead to the application of different food preservation methods. Drying is the most commonly applied stabilisation process to increase shelf life and can be defined as the transformation of the herbs from a dynamic watery living plant to a more stable dry state. It is an effective method of preservation that inhibits the growth of microorganisms and delays the onset of some biochemical reactions, but it may affect the quality of the final product [1, 2]. Hot-air drying causes heat damage to the plant tissue and severely modifies the physical and chemical characteristics of the marketed product. Freeze drying is usually applied to avoid heat damage, but even in this case the loss of quality parameters is visible [3].

The quality of herbal products must be compared with the physical and chemical properties of the original herb [1]. Therefore a high quality herb product is characterised by a natural colour, a high intensity of the characteristic aroma and an absence of an off-flavour.

The visual changes in colour from green to olive brown in processed herbs represent a loss of quality. Chlorophylls, which are the pigments responsible for the green colour of plants, degrade during processing and storage. The chlorophyll degradation depends on temperature, pH-value, time, endogenous enzyme activity, oxygen and light [4, 5].

The typical aroma of herbs is due to the high content of essential oils. The essential oils consist of volatile flavour compounds, which are unstable and lead to a decrease in quality due to volatile losses and chemical reactions induced by heat. Especially high temperatures cause losses and changes in concentration of the volatile aromatic substances [6, 7, 8].

A considerable quality loss of dried herbs is connected with the formation of an off-flavour, which is perceptible as a taste and smell of hay. The substance 3-methyl-2,4-

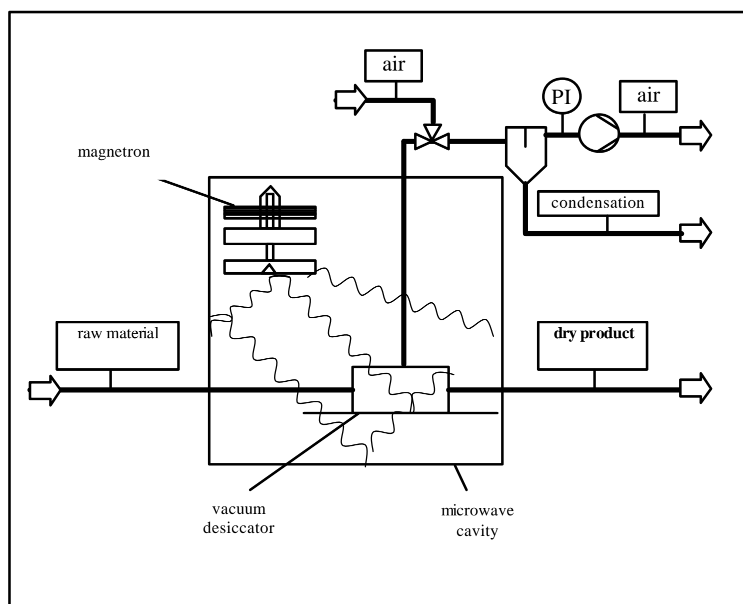

FIGURE 1 - Combined vacuum-microwave apparatus.

nonanedione is responsible for this hay-like off-flavour and is caused by peroxidation of furanoid fatty acids [9, 10]. This means that drying may lead not only to a loss of colour and typical flavour compounds, but also to the formation of volatile compounds causing hay-like off-flavours.

Many studies have been made on the quality improvement of dried herb products. However, most publications deal with the combination of unit operations, and the evaluation of the final product is usually focused on the stabilisation of single quality parameters [8, 11 - 15]. The present study involves the investigation of a complete quality stabilisation of fresh herbs, which can be achieved using a combined vacuum-microwave drying (VMD) process in comparison to hot-air drying (HAD).

MATERIAL AND METHODS

Plant source

Plain leaf parsley (*Petroselinum crispum*), was obtained from a local sweet herb cultivator. In all experiments, only the leaves of the freshly harvested plant were used, excluding the stems. The initial moisture content of the plant material was measured at 77 % on a wet weight basis.

Drying

Vacuum-microwave drying (VMD)

Figure 1 schematically depicts the experimental apparatus (Micromat 328 L, AEG, modified) for combined vacuum-microwave drying.

Drying parameters

Microwave power:
- 642 W (calculated according to Schiffmann [16])
- applied in a pulsed manner

Vacuum conditions (maintained by vacuum pump):
- during power on-time: 40 mbar
- during power off-time: 10 mbar

Final water content: <10 %

Conventional hot-air drying (HAD)

Fresh parsley leaves from the same source used in the preceding drying method were dried using a laboratory hot-air oven (T 5050, Heraeus).

Drying parameters

Drying temperature: 75 °C
Drying time: 50-70 min
Final water content: <10 %

Water content

Water contents of fresh and dried samples of parsley leaves were determined (in duplicates) using a laboratory oven at 103 ± 2°C, according to Masanetz [10]. The samples were dried to a constant weight (3 h) and the water content was calculated from the difference between the wet and the dry weights.

Colour

A 0.1 g sample of dried parsley leaves was filled into a bottle (SCHOTT) and homogenised with 100 mL of acetone-water (80:20 v/v) for 2 min. using an Ultra-

Turrax TP 18 / 2 from Janke & Kunkel KG. Afterwards the pigments (chlorophylls) were extracted from the plant material by shaking the closed bottle for 24 h under light exclusion at room temperature. The greenish hued mixture was vacuum filtered through filter paper (Schleicher & Schuell, 595, ∅ 70 mm). Objective colour measurements of the filtrate were made with a Chroma-Meter CR310 colourimeter (Minolta). The apparatus was calibrated with a standard white tile (standard white No. 21133006). A volume of 30 mL of the filtrate were transferred into a 10 cm-diameter glass dish and placed on top of the light source of the colourimeter. The dish was covered with a black plate to avoid influence of daylight variations and then measured. The results were expressed as CIE-Lab colour values L* (whiteness / darkness), a* (red / green) and b* (yellow / blue). For evaluation of the green colour impression only the a*-values were taken into consideration.

Essential oil content

Essential oils are volatile oils which are responsible for the characteristic aroma of herbs. Generally these oils are mixtures of a multitude of volatile substances. Preserving the whole content of essential oils is accompanied with the retention of the typical aroma of herbs. The essential oil content of herb samples was determined according to ISO 6571 [17]. The distillation method consists in distilling an aqueous suspension of the plant material and collecting the distillate in a graduated tube containing a measured volume of decahydronaphthalene to absorb the volatile oil. After separating the organic from the aqueous phase the total volume of the organic phase is read and the essential oil content is calculated by subtracting the volume of decahydronaphthalene.

Aroma rating (odour profile)

The aroma rating of fresh and dried parsley was carried out by a trained sensory panel of 10 persons (training of panellists according to DIN 10961, part 2) [18]. Each chopped sample (0.2 g each) was placed in a sealed beaker. Samples were stored for 30 min. at room temperature and, after removing the lid, sniffed by the panellists, who were wearing green goggles to avoid any influence of the different sample appearance on the aroma rating. The panellists rated the odour impressions “parsley-like”, “green-grassy”, and “hay-like / straw-like” on a intensity scale of 0 (not perceptible) to 6 (very strong perceptible) in order to describe the odour profile. Also the general impression was rated on a category scale of 0 (unpleasant) to 6 (pleasant). All results obtained by the panellists were averaged.

RESULTS AND DISCUSSION

The evaluation of results must consider the main quality parameters, namely colour, aroma, and off-flavour for an investigation of a complete quality improvement study of dried herb products.

A significant colour improvement can be achieved by using a vacuum-microwave drying process for producing dried herbs. Higher negative “a*-values” indicate a greener colour in the CIE-L*a*b*-colour measurement system. The initial a-value directly after drying is higher for vacuum-microwave dried samples (-7.4) than for hot-air dried (-6.7) parsley as can be seen in Figure 2. Also, the colour retention over 8 weeks-stored in darkness is significantly higher by using a vacuum-microwave drying process in comparison with convective drying. Colour degradation was observed in both samples, but was faster for hot-air dried parsley. The drying temperature has a great influence on the colour stability. Dielectric drying is carried out at room temperature preserves the green colour of the plant material. This is in accordance with Krokida et al. [19], who showed the effect of drying temperature on colour changes by drying fruits and vegetables. High temperatures in the range of 50-90 °C caused high degradation of natural dyes. Hayawaka and Timbers [20] investigated the influence of heat treatment on the changes in visual green colour of some vegetables and pointed out, that the degradation of chlorophylls is depending on processing time and temperature. Therefore the drying time has a favourable effect on the colour retention of the final product. The rapid energy transfer of microwave heating generates very rapid, low-temperature drying and therefore the drying process is shorter in time [14, 15, 21].

A visible colour improvement of dried herbs can be obtained, but the coherence of final water content with colour stability must be considered. Figure 3 represents the colour degradation of VMD-parsley with two different moisture contents in comparison with HAD-sample stored under UV-light irradiation. The VMD-samples had higher water contents (8.1 % and 10.8 %) than air-dried parsley (4.2 %). The initial a-value was higher for both VMD- (-7.4 and –6.9) compared to HAD-parsley (-6.7), but the degradation in green colour was faster. Comparing the two VMD herb products, it can be seen, that the colour degradation increases with a higher water content. That means, that the final water content is obviously decisive of less colour retention. LaJollo et al. investigated the chlorophyll degradation in dried spinach in dependence on a_w-value. It was shown, that chlorophyll is degraded under a_w-value of 0.32 to a less degree. This a_w-value or even lower should be reached for an extensive chlorophyll stability [22, 23]. The VMD-samples had a calculated a_w-value of 0.35 (8.1 %) and 0.43 (10.8 %) as determined with the help of a sorption isotherm of Rieblinger [24]. In contrast, HAD-parsley had a calculated a_w-value of 0.11 (4.2 %). The effect of water content on the deterioration of the chlorophyll pigment was also observed from ELBE. This study confirmed, that the rate of chlorophyll degradation increases with an increase in a_w-value of the system. In dehydrated foods, deteriorative reactions depend on the state of the water present. At high water activities enzymatic reactions and growth of micro-organisms take place and at lower a_w-values ($a_w \leq 0.25$) the degradation velocity tends to a minimum [25].

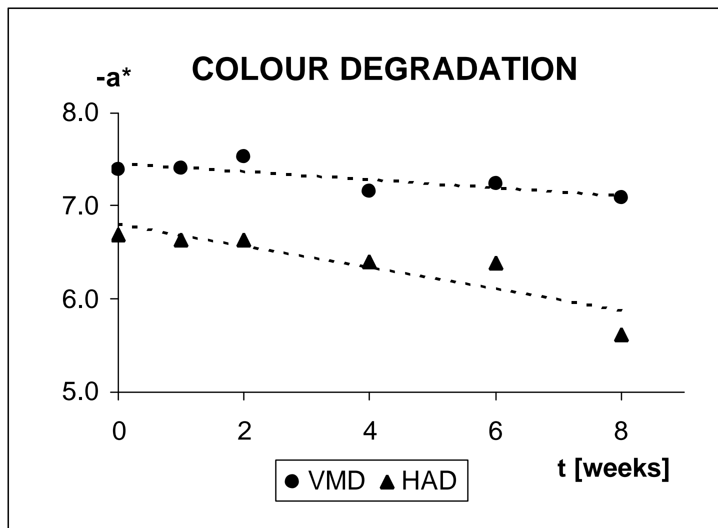

FIGURE 2 - Colour degradation of vacuum-microwave dried (VMD) and hot-air dried (HAD) parsley during storage in darkness at room temperature.

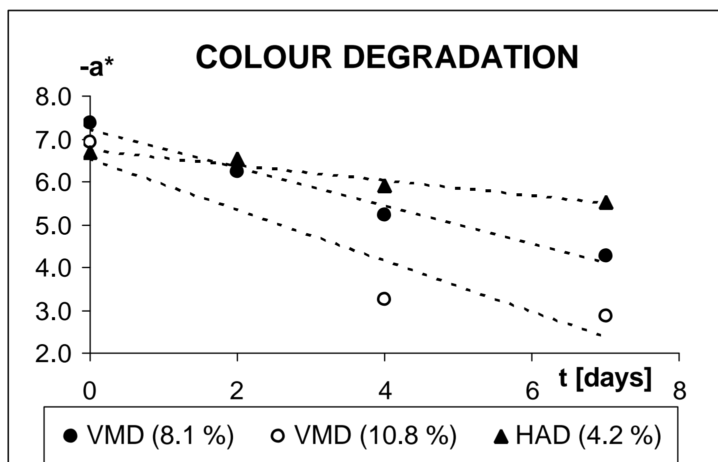

FIGURE 3 - Colour degradation of vacuum-microwave dried (VMD) parsley with different water contents [%] and hot-air dried (HAD) parsley stored under UV-light irradiation.

Herbs are used to flavour foods due to their steam distillable content of essential oils. Johnson showed that evaporation and loss of volatile aromatic substances occur at temperatures higher than 40 °C [6]. Drying in this temperature range or even below takes several hours and is industrially not feasible. Preservation of the essential oils of herbs is an important quality factor, and determination of its content gives information about the changes of aroma and flavour volatiles during processing, especially drying. Figure 4 represents the content of essential oils of dried herbs in comparison with fresh herbs. It can be seen that the preservation of the essential oils of vacuum-microwave dried parsley with 93.9 % is much higher compared to the convective-dried parsley with 30.0 %. As expected, hot-air drying caused a significant loss of aroma in contrast to vacuum-microwave drying. Reason for this considerable loss during hot-air drying is the high temperature of the heat transfer medium. The applied vacuum allows water to vaporize at a lower temperature and faster rate than at atmospheric pressure [26]. Heindl [13] investigated the drying of valerian roots. VMD preserved 82-94 % of the essential oils. Convective drying at a temperature of 50 °C enabled achievement of only an aroma preservation of 45 %. Regier et al. [27] and later Erle [12]

also achieved a high aroma preservation using microwaves for drying parsley. It was explained, that essential oils exist in small droplets and form a separate phase. This oily phase has a higher boiling point than water and its loss is less during the fast evaporation of water using dielectric drying. Therefore, herbs can be dried without exposure to high temperature. Another possible effect might be the mechanisms of microwave heating. The electromagnetic field causes dipolrotation of permanent dipoles like water molecules and ionic polarisation, thus heating the material. Grüneberg et al. [28] investigated the microwave heating of edible fats and oils and found out that the dielectric properties of foods influence the heating behaviour. It is conceivable, that the heating of essential oils is less than water using microwaves. The different heating behaviours of the two phases is in case of drying herbs profitable, which can be defined as a selective heating preserving a high content of essential oils.

One of the main sensory quality parameters of dried herb products is the hay-like off-flavour. The avoidance of this sensory impression is substantial for the evaluation of the final product. The results of the olfactory study of different dried parsley in comparison with fresh parsley is presented in Figure 5. The aroma rating in form of characterisation of odour profiles allows a qualitative evaluation of the aroma of herbs. Both fresh and dried parsley were rated for the odour impressions, parsley-like, green-grassy, and hay-/straw-like by a trained sensory panel. Furthermore, the general impression of the odour was rated and describes the consumer acceptability. Fresh parsley was used as a reference substance. As can be seen in the aroma rating, the odour impression parsley-like was rated 5.5 (strong perceptible), green-grassy with 4.1 (noticeable perceptible) and the general impression was 5.5 (pleasant) for fresh samples. As expected, the hay-/straw-like off-flavour was not perceptible in fresh parsley. All dried

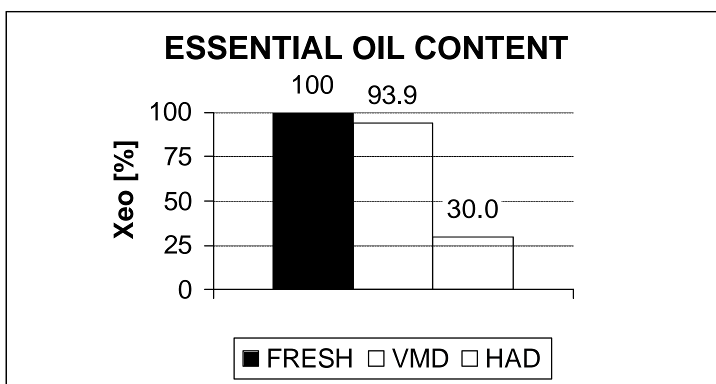

FIGURE 4
Essential oil content X_{eo} [%] of vacuum-microwave dried (VMD) and hot-air dried (HAD) parsley relating to fresh parsley.

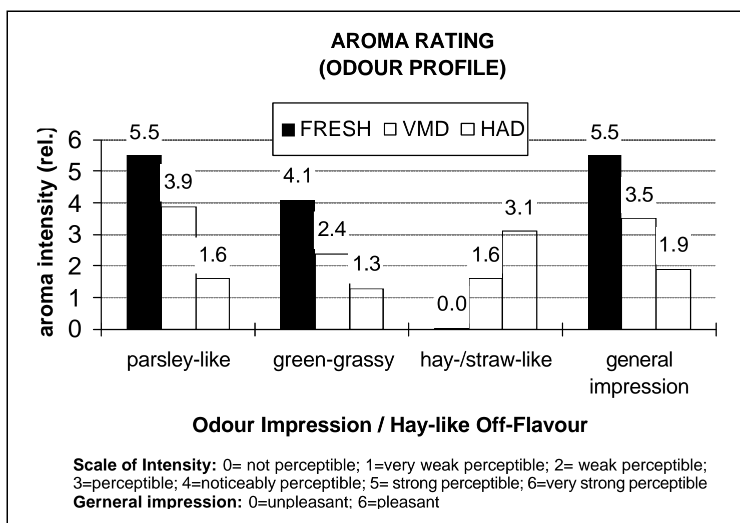

FIGURE 5
Aroma rating of fresh, vacuum-microwave dried (VMD) and hot-air dried (HAD) parsley.

samples were rated with a less aroma intensity for parsley-like. The VMD-parsley was rated with 3.9 (noticeably perceptible) and the hot-air dried sample only with 1.6 (weak perceptible). The odour impression parsley-like was better preserved by using vacuum-microwave drying. The grassy-green impression decreased strongly during drying. Green odorants were weak perceived in VMD-parsley (2.4) and very weak perceptible in hot-air dried parsley (1.3). It was shown, that there is a high loss in typical parsley flavour notes using hot-air drying. Furthermore, all dried samples had a hay-/straw-like off-flavour, but with different intensities. The off-flavour defect was weak perceived in VMD-sample (1.6) and was clearly perceived in HAD-parsley (3.1). Masanetz [9, 10, 29] investigated the aroma profile of dried spinach and parsley and explained which substances are responsible for the off-flavour impression formed during drying and storage. The investigation of hot-air dried parsley revealed, that the parsley-like notes decreased strongly during processing. On the other hand, the hay-like off-flavour strongly increased and adversely affected the odour impression. That investigation confirms the results of this study. Summarised, it can be said that the drying causes a change in the aroma profile through the change of concentration of odour notes of typical parsley flavour. The odour impressions parsley-like and grassy-green decrease. Moreover, as a result of heat treatment during hot-air drying, aroma affecting substances can be perceived, which adversely affect the typical parsley flavour. The hay-like off-flavour defect is caused by an oxidative degradation of furanoid fatty acids leading to 3-methyl-2,4-nonanedione [9]. In conclusion, drying always affects the aroma quality and, therefore, the general odour impression of herbs and the odour always differ from the one of fresh parsley (5.5). Remarkable is the aroma preservation achieved using vacuum-microwave drying in comparison with the conventional hot-air drying. The general impression of the odour of dried parsley was 3.5 for VMD and only 1.9 for HAD. Further, using VMD reduces the formation of hay-like off-flavour substances. The absence of air during drying may inhibit oxidation reactions and prevent the off-flavour formation to a great extent [3].

CONCLUSIONS

The quality of dried herbs depends on the drying method and the drying conditions used. Vacuum-microwave drying results in a better product quality than the conventional hot-air drying. VMD-parsley was greener in colour, exhibited a higher content of essential oils and less hay-like off-flavour defects than those prepared by HAD. The improved quality stabilisation using VMD is mainly caused by low drying-temperatures, fast heat and mass transfer, and absence of air during drying. In general, VMD is a suitable dehydration method to produce high-valuable dried herbs.

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MICROBIAL COMMUNITY DYNAMICS DURING RIPENING OF FERMENTED SAUSAGE PRODUCED IN APULIA REGION (ITALY)

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SUMMARY

Microbial changes during meat fermentation (salami), made without commercial starter cultures, were followed at two artisanal factories, A and B, in Apulia region (South Italy). Fermentation was governed by an active lactic flora (> 10^7 CFU g^{-1}) and salt tolerant, catalase positive cocci (> 10^5 CFU g^{-1}). Total enterobacteria decreased from $4.5x10^3$ CFU g^{-1} (0 day) to $2.2x10^1$ CFU g^{-1} (28th day) in salami of the factory A, where the terminal values of pH and a_w were 5.0 and 0.86, respectively. In the salami produced in factory B, *Enterobacteriaceae* showed a minor decrease [from $4.8x10^4$ (0 day) to $1.2x10^3$ (28th day)] probably due to minor decreases in pH (5.6) and a_w (0.87) at the end of ripening (28th day). Enterococci exceeded 10^5 CFU g^{-1} at the end of ripening (28th day). The microbiological significance of each bacterial group was discussed.

KEYWORDS: Sausage, microbial community.

INTRODUCTION

Sausage manufacture is considered to occur in three phases: formulation (mixing of ingredients), fermentation and ripening [1, 2].

In Europe the predominant fermented meat products are Italian salami and German Rohwürste (raw sausage) with numerous regional variants. According to Adams [3], the annual European production of these products is around $0.5x10^6$ tonnes, with an average *per capita* consumption of 2 Kg. Germany is responsible for almost 50% of this annual production and the *per capita* consumption in this country is about 3.9 kg, almost 6% of the total meat consumption [4].

The manufacture of fermented sausage has a long tradition in Italy, where there is a wide variety of typical salami preparations [5, 6]. In Apulia region in South Italy some typical fermented meat products are still produced with traditional technologies without selected starters. These traditional sausages, which undergo an adventitious fermentation, are often of superior quality compared to those inoculated with starters and produced on industrial scale. The phenomenon is attributed to the specific composition and metabolic activity of the indigenous microflora [7-9]. However, the use of starter cultures for sausage production is becoming increasingly necessary to guarantee safety and standardize product properties, including consistent flavour, colour and shorter ripening time [10]. To achieve this, the prevailing species involved in indigenous sausage fermentations should be determined, and well-adapted strains further selected on the basis of the most interesting biochemical and technological properties [7, 11, 12].

The aim of this preliminary study was to describe the bacterial diversity during natural fermentation of Apulia region sausages. In a future work the characterization of biochemical and technological performance of several strains of lactobacilli and micrococci, isolated from these salami and used as starter cultures, will be presented.

MATERIALS AND METHODS

Preparation of sausage

The formulation of sausages was conducted in two meat products factories, A and B, in Apulia region, under the traditional conditions normally applied there. The following ingredients were employed (%): pork meat, 75; pork back fat, 25; salt, 2.5; sugars (glucose and lactose), 2; mixed spices (black pepper, cloves and garlic), 0.4; potassium nitrate, 0.02; sodium nitrite, 0.02; sodium ascorbate, 0.05.

Initially the meat was at –10 °C and was ground through 5 and 3 mm dies. The mixture temperature after grinding was –2 °C. Once all the ingredients had been added, the mixture was placed in steel containers and held in cold storage for 48 hrs at 0 °C. Furthermore, the mixture was filled into 45 mm synthetic casing. After filling the product was dried in a fermentation chamber for 7 days. During this period, the temperature and relative humidity (RH) were gradually decreased from 25 °C and 92% RH to 18 °C and 85% RH. Thereafter, curing was carried out in a controlled temperature-room at 13-14 °C with 80% RH. Total curing time was 4 weeks, after which the salami were ready for consumption.

Sampling

Three batches of sausage prepared as described above at each A and B plant were analysed at 0 (after filling), 2, 7, 14 and 28 days after formulation. Sausages were transported to the laboratory and subjected to microbiological and physio-chemical analyses. The samples were kept refrigerated (2 °C) until analysis, normally within 4 hrs of delivery.

Microbiological analysis

After removing the sausage casing, 20 g of the sample were homogenized in 180 ml sterile saline peptone water (8.5 g NaCl per litre, 1 g of bacteriological peptone per litre) in a Colworth Stomacher (London) for 1 min at low speed and 2 min at high speed at room temperature. Decimal dilutions were prepared and the following analyses were carried out in duplicate agar plates:

a) aerobic mesophilic counts (AMC) were determined on Plate Count Agar (PCA, Oxoid, Gabagnate Milanese, Italy), incubated at 32 °C for 72 hrs;
b) lactic acid bacteria (LAB) on de Man, Rogosa, Sharpe (MRS, Oxoid) incubated at 32 °C for 72 hrs in Atmosphere Generation System (Anaerogen, Oxoid);
c) salt tolerant, catalase positive cocci (micrococci-staphylococci) on Mannitol Salt Agar (MSA, Oxoid), incubated at 37 °C for 48 hrs;
d) total enterobacteria on Violet Red Bile Glucose Agar (VRBG, Oxoid), incubated at 37 °C for 24 hrs;
e) enterococci on Slanetz and Bartley Agar (SB, Oxoid), incubated at 37 °C for 48 hrs.

Measurement of pH and water activity

The pH of each sample of sausage was determined with an Orion Research digital pH-meter by inserting the electrode directly in each sample. A_w measurements were carried out using a Rotronic-Hygrometer (AW-WIN, PBI International). Water activity was measured in 3 slices (5 g each) of the sausage sample.

RESULTS AND DISCUSSION

The microbial changes during fermentation and ripening of traditional salami, produced in A and B factories are presented in Tables 1 and 2, respectively. It can be observed that in factory A the initial counts of the aerobic mesophilic flora, lactic acid bacteria and salt tolerant cocci were higher than in factory B. This feature was probably due to the variation in the natural existing microflora on the batches of meat used.

LAB became predominant (Tables 1 and 2), as they increased from 2.2×10^5 (0 day) to 9.8×10^8 CFU g^{-1} (7th day) in the factory A and from 2.5×10^4 (0 day) to 4.0×10^7 CFU g^{-1} (7th day) in factory B. After the 7th day, lactic acid bacteria remained almost constant up to day 28 (Tables 1 and 2) in both factories. AMC on PCA seemed to follow the same trend as lactobacilli counts on MRS agar.

TABLE 1
Microbiological changes (CFU g^{-1})[a] during ripening of traditional Apulia region salami produced in factory A.

Time of ripening	PCA[b]		VRBG		SB		MRS		MSA	
(days)	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
0	2.1×10^7	8.2×10^6	4.5×10^3	4.5×10^2	4.5×10^2	1.4×10^2	2.2×10^5	3.5×10^4	1.4×10^4	3.3×10^3
2	4.0×10^7	4.1×10^6	5.4×10^3	5.6×10^1	4.5×10^2	7.1×10^1	3.1×10^5	2.6×10^5	1.0×10^4	1.1×10^3
7	9.1×10^7	4.9×10^6	1.5×10^3	4.0×10^2	4.5×10^4	3.9×10^4	9.8×10^8	3.2×10^8	1.5×10^6	1.7×10^6
14	1.2×10^8	7.1×10^5	7.2×10^1	6.7×10^1	6.2×10^5	8.3×10^5	1.0×10^9	1.1×10^8	3.4×10^6	3.8×10^5
28	8.7×10^8	2.4×10^7	2.2×10^1	1.1×10^1	6.6×10^5	6.4×10^5	1.1×10^9	1.5×10^8	1.6×10^6	6.5×10^5

[a] Each number is the mean of two sausages samples taken from three batches produced in factory A
[b] Symbols: PCA, Plate Count Agar; VRBG, Violet Red Bile Glucose agar; MRS, de Man, Rogosa, Sharpe agar; SB, Slanetz and Bartley agar; MSA, Mannitol Salt Agar; Sd, standard deviation

TABLE 2
Microbiological changes (log CFU g^{-1})[a] during ripening of traditional Apulia region salami produced in factory B.

Time of ripening	PCA[b]		VRBG		SB		MRS		MSA	
(days)	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
0	$2.6x10^5$	$8.4x10^4$	$4.8x10^4$	$9.9x10^3$	$9.2x10^2$	$5.3x10^2$	$2.5x10^4$	$2.0x10^4$	$3.0x10^3$	$1.5x10^4$
2	$8.8x10^7$	$2.2x10^6$	$5.5x10^5$	$1.3x10^5$	$7.2x10^4$	$6.6x10^4$	$6.3x10^7$	$3.6x10^7$	$1.1x10^7$	$4.0x10^6$
7	$1.7x10^7$	$4.5x10^7$	$1.4x10^5$	$1.3x10^5$	$1.4x10^5$	$7.1x10^4$	$4.0x10^7$	$4.6x10^7$	$1.8x10^6$	$6.3x10^5$
14	$3.3x10^7$	$4.2x10^6$	$1.8x10^4$	$3.5x10^3$	$3.2x10^5$	$1.4x10^4$	$3.8x10^6$	$3.1x10^7$	$8.9x10^7$	$1.5x10^6$
28	$1.1x10^7$	$1.7x10^7$	$1.2x10^3$	$9.6x10^2$	$2.4x10^4$	$1.7x10^4$	$4.2x10^7$	$4.9x10^7$	$3.2x10^5$	$4.2x10^5$

[a] Each number is the mean of two sausages samples taken from three batches produced in factory B
[b] Symbols: PCA, Plate Count Agar; VRBG, Violet Red Bile Glucose agar; MRS, de Man, Rogosa, Sharpe agar; SB, Slanetz and Bartley agar; MSA, Mannitol Salt Agar; Sd, standard deviation

TABLE 3 - Changes in pH and Aw during ripening of traditional salami produced in Apulia region.

Time of ripening	Factory A				Factory B			
	pH		Aw		pH		Aw	
(days)	Mean[a]	sd	Mean	sd	Mean	sd	Mean	sd
0	5.8	0.03	0.95	0.03	5.6	0.04	0.94	00:03
2	5.7	0.04	0.94	0.01	5.1	0.13	0.93	00:03
7	5.5	0.06	0.93	0.01	4.9	0.06	0.89	00:03
14	5.4	0.03	0.86	0.01	4.8	0.07	0.87	00:01
28	5.0	0.07	0.86	0.03	5.6	0.14	0.87	0.01

[a] Each number is the mean of two sausages samples taken from three batches produced in factories A and B

The role of LAB in the fermentation of cured raw meat products is supposed to be very important. The lactic acid bacteria are essential agents of fermentation, as the inherent stability of the product is primarily dependent on conversion of sugars into acid [13, 14]. They are responsible for the drop in pH, which, in turn, can inhibit the growth of spoilage and pathogenic microorganisms [15]. LAB also affect flavour and texture [14]. For instance, isopeptidase activity from lactobacilli species associated with meat products may be involved in proteolytic phenomena generating small peptides and free amino-acids involved in flavour development [16].

Micrococcaceae counts indicated that bacterial population started at an initial level of c.a. 10^4 CFU g^{-1} in both factories, but in the middle of drying stage (14th day) they reached values of $3.4x10^6$ CFU g^{-1} (factory A) and $8.9x10^7$ (factory B) (Tables 1 and 2). Salt tolerant cocci are used in combination with LAB due to their ability either to contribute to flavour mainly through lipolytic activities or to reduce nitrate and produce catalase [17]. However, recent works indicate that staphylococci have little lipolytic activities under conditions normally found in the sausage [18, 19], although these bacteria could be responsible for lipolysis in the early stages of ripening when conditions of temperature, pH and NaCl% would be more favourable.

Total enterobacteria, present at casing at levels of $4.5x10^3$ CFU g^{-1} and $4.8x10^4$ CFU g^{-1}, respectively, in A and B factories, were detected at very low levels ($2.2x10^1$ CFU g^{-1}) after 28 days of ripening in salami from the factory A, but at high level ($1.2x10^3$ CFU g^1) in salami from the factory B. The decrease in salami from the producer A was correlated with the presence of other metabolic compounds responsible for their inhibition [20, 21].

In contrast, enterococci increased after 7 and 2 days in salami produced, respectively, from factories A and B; they remained at a level above 10^4 CFU g^{-1} until 28th day. Although, enterococci enhance sausage aroma and taste by proteolytic activities, it can also compromise safety if opportunistic pathogenic strains proliferate [9, 22].

The pH and a_w values (pH 5.0 and 5.6; A_w 0.86 and 0.87) (Table 3) from A and B producer, respectively, were related to the final number of LAB and to dehydration of the sausage. These values prove the rapid and correct ripening of salami examined. However, although no sensory evaluation was conducted in this study, the sensory properties of the ripened sausages varied greatly between the manufacturers A and B, or batches from the same artisanal manufacturer.

Work is now in progress in order to select "wild" strains from the microflora which govern the complex microbiological process of ripening; the aim is to develop a combination of bacterial cultures (LAB and salt tolerant cocci). These starters may be helpful to obtain an improvement in the quality and safety of artisanal salami.

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SEARCH OF VEROCYTOTOXIGENIC *Escherichia coli* (VTEC) IN FOODS OF ANIMAL ORIGIN

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SUMMARY

Hundred and forty samples of different foods of animal origin were examined to verify their hygienical conditions (total microbial count, total coliforms count, faecal coliforms count) and to recover possible verocytotoxigenic *Escherichia coli* (VTEC), with particular attention to the serotype O157:H7.

Two hundred and seventy *E. coli* strains were isolated, but none of these belonged to the VTEC group demonstrating that verocytotoxigenic *E. coli* is not very often present in Italian foods of animal origin.

KEYWORDS: *Escherichia coli*, verocytotoxigenic *Escherichia coli* (VTEC) group, foods of animal origin.

INTRODUCTION

Escherichia coli is a common inhabitant of the human large intestine. It also lives in the intestine of many wild as well as domestic animals. In the most cases this bacterium does not show pathogenic characteristics, nevertheless, four principal groups of pathogenic *E. coli* have been recognized: ETEC (enterotoxigenic), EPEC (enteropathogenic), EIEC (enteroinvasive) and EHEC (enterohemorrhagic). EHEC serotypes include the VTEC *E. coli* (verocytotoxin producers). In 1983 [1] for the first time the pathogenicity of the serotype VTEC O157: H7 was demonstrated and in the following years several cases of foodborne outbreaks of it were indicated in different areas of the world [2-6].

Different kinds of foods of animal origin are amongst the most common vehicles of VTEC *E. coli* infection [7-10]. In the light of these findings and considering VTEC as a very interesting emerging pathogen, the aim of the present work was to check the microbiological situation of different kinds of food, possible transmission routes of VTEC serotypes and to recover possible verocytotoxin-producing *E.coli* (VTEC; serotype O157: H7) strains.

MATERIALS AND METHODS

Samples

Hundred and forty samples of different kinds of food were examinated in the present work (bovine meat: 60 samples; raw bovine milk: 40 samples; fresh cheese: 20 samples; confectionery cream: 20 samples). The samples were collected from producers and retailers in Puglia and Basilicata (Italy).

Microbiological Analyses

Total microbial count (TMC), total coliforms count (TCC) and faecal coliforms count (FCC) were performed to check samples' hygienical conditions.

10 g aliquots of each sample was diluted in 90 ml sterile peptone water (0.1% w/v) and blended with a Stomacher Seward 400. Each sample was decimally diluted and spread on specific media: Plate Count Agar (PCA; Biolife, Milan, Italy) to recover TMC after incubation for 48-72 hrs at 32 °C; Brilliant Green Bile Broth (BGBB; Biolife) and tryptone water (TW; Biolife), to determine TCC and FCC according to the Most Probable Number (MPN) method.

For the detection of *E. coli* serotype O157: H7 10 g of each sample was diluted in 40 ml sterile saline plus 1% tryptophan, blended (Stomacher Seward 400) and incubated for 3 to 6 hrs at 37 °C. Each dilution was then spread on Sorbitol McConkey Agar (SMCA; Biolife) and incubated at 37 °C for 18-24 hrs. After incubation typical colonies were Gram-stained, identified with Api 20E (BioMerieux, France) and tested with inverse passive latex agglutination test (Oxoid).

RESULTS AND DISCUSSION

In Tables 1-4 the percentages of samples with sorbitol-negative colonies, at least one *E. coli* colony and strain-positive to latex test are shown. Confectionery cream and raw meat have the highest percentages of sorbitol-negative colonies, in particular 84.35% of cream

samples and 68.62% of meat samples, while fresh cheese samples were the most contaminated by *E. coli* (85.86 %). These results are in agreement with Soncini et al. [11] and d'Aubert et al. [12], especially the data regarding fresh cheese. D'Aubert and co-workers [12] have demonstrated the important role of this product as a risk factor of the possible diffusion of VTEC. The present work does not reveal the presence of VTEC strains in fresh cheese. Perhaps good hygienical conditions depend on both the milk quality and handling.

Table 5 presents the percentages of three different types of bacterial colonies recovered from the products examined. The most 4-methylumbelliferyl-D-glucuronide (MUG) and sorbitol negatives were found in raw milk (58.31 %). These strains were identified as *Proteus* spp., *Enterobacter* spp. and *Hafnia alvei* according to d'Aubert et al. [12]. It strongly confirms what already has been reported about fresh cheese, that the most MUG and sorbitol positives were significantly recovered in this product (85.86 %). It must be emphasized that 34 % of them were identified as *E. coli.* (data not shown). However, none of the *E. coli* strains isolated was positive to latex test. This fact is in accordance with Conedera et al. [7] who did not recover any serotype O157: H7 from 144 meat samples, even though they pointed out the presence of verocytotoxin in 9% of the samples examined. Also Soncini and Valenti [13] did not recover any VTEC strain in a study on dairy products.

It is important to note that a different analytical procedure including an enrichment step is not necessary to recover VTEC strains, in fact, for example, Soncini et al. [11] isolated two VTEC *E. coli* without selective enrichment.
In conclusion, in the light of two *E. coli* outbreaks recorded in 1992 and 1993 in Italy [3], the VTEC diffusion problem must be considered also as an Italian one and our findings revealed that some products, i.e. fresh cheese, are a possible risk source. Therefore, a strict control in every step of transformation process and trading the product is important, even if Italy seems to be an area where VTEC risk is not wide-spread.

TABLE 1 - Raw meat samples (no. of samples 60).

A	Samples with sorbitol negative colonies	68.62 % [a]
B	Samples with at least one *E. coli* colony	31.38 % [b]
C	Samples positive to latex test	-

[a] 29.3% of 140 samples examined
[b] 13.5% of 140 samples examined

TABLE 2 - Raw milk samples (no. of samples 40).

A	Samples with sorbitol negative colonies	74.03 % [a]
B	Samples with at least one *E. coli* colony	25.97 % [b]
C	Samples positive to Latex Test	-

[a] 21% of 140 samples examined
[b] 8% of 140 samples examined

TABLE 3 - Fresh cheese samples (no. of samples 20).

A	Samples with sorbitol negative colonies	14.14 % [a]
B	Samples with at least one *E. coli* colony	85.86 % [b]
C	Samples positive to Latex Test	-

[a] 2% of 140 samples examined
[b] 12.1% of 140 samples examined

TABLE 4 - Confectionery cream samples (no. of samples 20).

A	Samples with sorbitol negative colonies	84.35 % [a]
B	Samples with at least one *E. coli* colony	15.65 % [b]
C	Samples positive to Latex Test	-

[a] 12.1% of 140 samples examined
[b] 2% of 140 samples examined

TABLE 5 - Distribution (percentage) of different types of bacterial colonies in relation to their reaction with 4-methylumbelliferyl-a-D-glucoronide (MUG) and sorbitol (SOR) in different kinds of food of animal origin.

Products	**Colony type (%)**		
	MUG+[a] SOR+	MUG-[b] SOR-	MUG+ SOR-
Raw meat	25.97	58.31	15.71
Raw bovine milk	31.38	5.49	63.12
Fresh cheese	85.86	0.25	13.88
Confectionery cream	15.65	5.90	78.44

[a] positive reaction
[b] negative reaction

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THE EFFECT OF pH AND TEMPERATURE ON SURVIVAL OF *Shigella flexneri* STRAINS

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SUMMARY

The survival of *Shigella flexneri* strains NCTC 8192, NCTC 9729 and ISS 75 were determined in TSB broth as a function of low (from 3 to 6) pH and temperature (35, 21 and 4°C). Bacterial populations were determined periodically by aerobic plate counts. In general, survival increased as temperature decreased and as pH increased. Strain NCTC 8192 reached undetectable levels (< 1 log CFU ml^{-1}) at 35 °C in medium adjusted at pH 3.0 after 4 days, while strain 9729 survived at pH 3 and 3.5 until 2 and 6 days respectively. In the same condition (37 °C) strain ISS 75 reached undetectable levels at pH 3.0 and pH 3.5 after six and fourteen days, respectively. Strain NCTC 8192 was more susceptible than strains ISS 75 and NCTC 9729 to the acid environment (pH 3.0) at 21 °C. Furthermore, all the strains examined at 4 °C survived approximately for 6 days at lower pH (i.e. pH 3).

These results indicate that *S. flexneri* is acid resistant and that acidic foods, even stored at 4 °C may serve as vehicles of infection.

KEYWORDS:
Shigella flexneri, temperature, pH, survival.

INTRODUCTION

Shighella is recognized as an important causative agent of foodborne gastrointestinal disease such as bacillary dysentery or shigellosis [1]. *Shigella* species are nonmotile, oxidase-negative, Gram-negative rods and closely related to *Escherichia coli*, forming a single species based on their DNA homology [2].

The potential for illness due to consumption of contaminated foods is relatively high, since the infective dose of *Shigella* may be between 10 to 500 organisms [3].

It has been estimated that the number of foodborne illnesses in the United States is about 448,000 cases annually and that the actual number of foodborne outbreaks may be significantly underreported [4]. However, in foodborne shigellosis outbreaks, *Shigella* is rarely isolated from epidemiologically implicated food, and this may be due to the failure of the organism to survive in the food until it is analysed or to inadequate isolation or identification methodologies [5, 6]. Although considerable effort has been made to investigate its epidemiology and virulence [7], only few studies have been carried out on the effects of environmental conditions. The most extensive report regarding the relationship between environmental factors (such as pH and temperature), and *Shigella* survival, was presented by Fehlhaber [8]. The author showed that several strains of *S. flexneri* were able to growth in a range of temperature from 10 °C to 44 °C and in both acid (pH 5.04) and basic (pH 9.19) solutions. Recently, survival of *Shigella* at lower pH (from pH 2 to 5) has been documented [5, 9], suggesting that acidic foods may serve as vehicles for infection. Therefore, it is essential to determine the role that food ingredients, storage and environmental conditions may play in determining the fate of *Shigella* in food and its ability to cause infection.

The objective of the present work was to study the survival of *S. flexneri* strains NCTC 8192, NCTC 9729 and ISS 75, in a microbiological medium under variable conditions of pH and temperature. The data obtained may be useful in order to predict the behaviour of *Shigella* species in food.

MATERIALS AND METHODS

Microorganisms

S. flexneri strains NTCT 8192 and NTCT 9729 were obtained from the "Central Public Health Laboratory" (PHLS) of London (UK), while strain ISS 75 was kindly provided by the Istituto Superiore della Sanità (Rome,

Italy). The strain stock cultures were stored at –80 °C in cryovials (Nalgene) containing Trypticase Soy Broth (TSB, Biolife, Milan, Italy) supplemented with 10% (v/v) glycerol. In order to reach the stationary phase, thawed stock culture aliquots (0.1 ml) were added to 10 ml of TBS broth and incubated for 24 h at 37 °C.

Medium

The TSB was adjusted to pH 6.0, 5.5, 5.0, 4.5, 4.0, 3.5 and 3.0, with either HCL 1N or NaOH 1N. The medium was distributed in 100 ml into 250 ml flasks capped with foam plugs, and sterilized by autoclaving. Autoclaving of the media did not result in significant changes in pH. A model CRISON MICRO 2001 was used for pH determination.

Culture conditions

One ml of inoculum was added to 10 ml of TSB medium to give an initial population level of 10^6 to 10^7 CFU ml^{-1}. The media were incubated on rotary shaker at 4, 21, and 35 °C in order to simulate typical refrigeration, room-storage temperature and abuse temperature, respectively. A minimum of two trials was conducted for each variable combination-treatment with two replicates or flasks per treatment and trial.

Determination of microbial populations

Bacterial populations were enumerated immediately after inoculation and at different storage times. Appropriate dilutions were made in pH 7.0 physiological solution and pour onto duplicate plates of TSA medium (TSB plus 1.5 % agar, pH 7.0). The plates were then incubated for 24 h at 37 °C and the colonies were counted.

RESULTS AND DISCUSSION

Survival characteristics of *S. flexneri* strains NCTC 8192, NCTC 9729 and ISS 75 were monitored at three different temperatures (35, 21 and 4 °C), by inoculation of bacterial cells into TSB medium adjusted to different pH as reported in materials and methods section.

During incubation at 35°C and pH 3.0 the strains NCTC 8192 and NCTC 9729 declined to undetectable levels after 4 days but were still present at greater than 1 log CFU ml^{-1} after 48 hours (Table 1). In particular, the strain NCTC 8192 was still present after 28 days at pH 3.5, while, at the same pH, the strain NCTC 9729 was inhibited after 14 days. The strain ISS 75 reached undetectable levels at pH 3.0 and 3.5 after 6 and 14 days, respectively. Recently, the survival of *Shigella flexnery* 5348 at 37 °C and pH 3.0 for 8 days has been documented [5]. Therefore, our results confirm the ability of *Shigella* to survive in an extreme acid environment and that acidic food may be implicated as vehicles for infection.

After an initial growth at 21 °C (Table 2), the population level of strains ISS 75 and NCTC 9729 were stable at pH 6.0, but undetectable after 14 days at pH 3.5. A further reduction of pH value from 3.5 to 3.0 affected the population level of strains NCTC 9729, which was undetectable after 7 days, while the strain ISS 75 was almost unaffected. The strain NCTC 8192 was not recovered after 14 days and 4 days at pH 3.5 and pH 3.0, respectively. Consequently, the strain was more susceptible than the strains ISS 75 and NCTC 9729 to the acid environment at 21 °C.

TABLE 1 - Survival of *Shigella flexneri* strains ISS75, NCTC 8192 and NTCT 9729 at 35 °C in Tripticase Soy Broth adjusted to acid pH.

S.flexneri strains	pH	Days					
		0	2	4	6	14	28
ISS75	**6.0**	5.8[a]	7.9	6.5	6.2	6.2	5.7
ISS75	**5.0**	5.9	7.6	6.4	6.1	6.0	5.5
ISS75	**4.5**	5.8	5.9	6.5	6.2	6.2	5.7
ISS75	**4.0**	5.9	5.0	5.0	5.0	2.9	1.2
ISS75	**3.5**	5.8	3.9	3.5	1.8	ND[b]	ND
ISS75	**3.0**	5.7	2.9	1.5	ND	ND	ND
NTCT 8192	**6.0**	6.0	8.3	7.9	7.5	7.3	7.2
NTCT 8192	**5.0**	5.8	7.9	6.5	5.6	4.0	2.5
NTCT 8192	**4.5**	5.9	5.0	5.0	5.0	2.0	2.0
NTCT 8192	**4.0**	5.9	3.5	2.5	2.0	2.0	2.0
NTCT 8192	**3.5**	5.9	2.5	2.5	1.8	1.8	1.2
NTCT 8192	**3.0**	5.8	1.2	ND	ND	ND	ND
NCTC 9729	**6.0**	6.0	7.3	7.9	8.1	6.0	5.9
NCTC 9729	**5.0**	6.0	7.2	7.9	6.5	6.0	5.9
NCTC 9729	**4.5**	5.9	5.0	4.5	4.5	3.0	ND
NCTC 9729	**4.0**	5.9	4.3	4.3	3.5	3.0	ND
NCTC 9729	**3.5**	5.9	4.0	2.9	1.5	ND	ND
NCTC 9729	**3.0**	5.8	1.2	ND	ND	ND	ND

[a]Values are expressed as CFU ml^{-1};
[b]Not detectable

TABLE 2 – Survival of *Shigella flexneri* strains ISS75, NCTC 8192 and NTCT 9729 at 21 °C in Tripticase Soy Broth adjusted to acidic pH.

S.flexneri strains	pH	Days					
		0	2	4	6	14	28
ISS75	**6.0**	5.8[a]	6.6	6.9	6.9	6.8	6.7
ISS75	**5.0**	5.9	7.6	6.4	6.1	6.0	5.5
ISS75	**4.5**	5.8	5.0	5.0	5.0	4.2	ND
ISS75	**4.0**	5.9	5.0	5.0	4.0	ND[b]	ND
ISS75	**3.5**	5.8	4.9	4.5	4.0	ND	ND
ISS75	**3.0**	5.7	3.9	3.2	3.0	ND	ND
NTCT 8192	**6.0**	6.0	8.3	7.9	7.5	6.6	6.2
NTCT 8192	**5.0**	5.8	7.9	6.0	6.0	6.0	5.0
NTCT 8192	**4.5**	5.9	6.1	5.0	5.0	5.0	4.5
NTCT 8192	**4.0**	5.9	2.9	2.0	2.0	ND	ND
NTCT 8192	**3.5**	5.8	3.0	2.0	2.0	ND	ND
NTCT 8192	**3.0**	5.8	1.2	ND	ND	ND	ND
NCTC 9729	**6.0**	6.0	7.3	7.3	7.5	7.5	7.3
NCTC 9729	**5.0**	6.0	6.0	5.9	5.5	4.8	ND
NCTC 9729	**4.5**	5.9	4.5	5.5	5.5	5.4	5.3
NCTC 9729	**4.0**	5.9	4.0	4.0	3.8	3.8	ND
NCTC 9729	**3.5**	5.9	4.0	4.0	3.9	ND	ND
NCTC 9729	**3.0**	5.8	3.0	3.0	ND	ND	ND

[a]Values are expressed as CFU ml^{-1};
[b]Not detectable

A longer survival of *S. flexneri* was observed at 4 °C in TSB adjusted to pH values below 6. Indeed, all the strains examined survived for 28 days at pH 4.0 and six days at pH 3 (Table 3).

In a previous work, Fehlhaber [8] found a lower resistance of *S. flexneri* and *S. sonnei* to acid pH. However, it is well-documented that the acid resistance in *S. flexneri* is highly dependent on the growth phase [10]. Indeed, *S. flexneri* can survive for long periods time, even in acidic food, when stationary phase cells are inoculated [10].

TABLE 3 – Survival of *Shigella flexneri* strains ISS75, NCTC 8192 and NTCT 9729 at 4 °C in Tripticase Soy Broth adjusted to acidic pH.

S.flexneri strains	pH	Days					
		0	2	4	6	14	28
ISS75	**6.0**	6.0[a]	6.0	6.0	5.9	6.0	5.8
ISS75	**5.0**	5.9	6.0	6.0	6.0	5.9	5.9
ISS75	**4.5**	6.0	5.0	5.0	5.0	4.5	ND[b]
ISS75	**4.0**	5.9	5.0	5.0	4.6	1.8	1.2
ISS75	**3.5**	5.8	5.0	5.0	5.0	3.2	ND
ISS75	**3.0**	5.7	3.9	3.2	2.1	ND	ND
NTCT 8192	**6.0**	6.0	5.3	5.9	5.5	5.6	3.5
NTCT 8192	**5.0**	5.8	5.5	5.5	5.5	5.0	2.5
NTCT 8192	**4.5**	5.9	3.7	3.0	2.9	2.8	2.0
NTCT 8192	**4.0**	5.9	3.3	2.5	2.5	2.0	1.3
NTCT 8192	**3.5**	5.8	2.0	2.0	2.0	1.5	ND
NTCT 8192	**3.0**	5.8	2.1	2.3	1.5	ND	ND
NCTC 9729	**6.0**	5.9	6.0	6.2	5.8	5.0	4.6
NCTC 9729	**5.0**	6.0	5.7	5.6	5.5	5.4	2.7
NCTC 9729	**4.5**	5.9	5.7	5.5	5.5	5.4	2.5
NCTC 9729	**4.0**	5.9	5.5	5.2	5.1	4.5	1.8
NCTC 9729	**3.5**	5.8	5.5	5.4	5.3	3.2	ND
NCTC 9729	**3.0**	5.8	1.8	1.4	1.2	ND	ND

[a]Values are expressed as CFU ml^{-1};
[b]Not detectable

The findings of the present study confirm the previous results of Taylor and Nakamura [11]. They have recovered cells of *S. flexneri* inoculated in the stationary phase after 14 days at 4 °C in TSB at pH 3.5. Furthermore, it is known that exposure of a bacterial species to a moderately acidic environment may improve their resistance, when transferred to a more acidic environment. This phenomenon has been termed as "acid adaptation" and it may be important in food safety. Although no acid adaptation has been studied in our samples, it has been observed in *Escherichia coli* [12], *Listeria monocytogenes* [13] and *Salmonella spp*. [14].

It is recognised that the temperature is the major factor in the survival of *Shigella* spp at low pH values [5]. For example, *S. flexneri* was inactivated within 24 h at 37 °C in a nutrient broth adjusted to pH 3.5, while *Shighella dysenteriae* survived for more than 6 days at 4 °C in orange juice at pH 3.5 [11]. All the strains examined were able to survive at 4 °C for more than 20 days at pH 3.5-4.0, suggesting that foods of pH 4 or lower, even stored below room temperature, may permit survival of the organisms over long period of time in sufficient numbers to cause illness.

The enhanced survival of food pathogens in chilled acidic environment has been described, in different food systems, by other authors. Weagant et al. [15] recovered *E. coli* O157:H7 strains inoculated into commercial mayonnaise (pH 3.6) after up 35 days of storage at 7 °C. However, this strain was undetectable after storage of 72 h at 25 °C and pH 3.6.

In conclusion, survival of *S. flexneri* in TSB increased as temperature decreased and as pH of the medium increased. Moreover, the data obtained, highlight new evidences on the ability of *Shigella* spp. to survive in acidic foods. In conjunction with other experiments currently being carried out in our laboratory, the data obtained will be used to develop a simple model in order to predict the fate of *Shighella* spp. in food.

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NUCLEI OF BOVINE TISSUES AS A SINK FOR FLAVANOLS AND FLAVONOLS

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SUMMARY

Special tissues of bovines were sampled in order to investigate the sink capacity of nuclei for flavanols (catechins) and flavonols (quercetin derivatives). Tissue sections of brain, intestine, kidney, liver and spleen were incubated for 3 h in a saturated solution of epigallocatechin-gallate. In the case of liver and spleen the entire flavanol group of grape wine seeds was used additionally. The flavanols were found to enter the cells and to associate particularly with the nuclei, which could be verified with the DMACA reagent giving a blue colour complex. Flavonols associated with spleen nuclei stained a yellow or green fluorescence with “Naturstoff reagent A”. Flavonoids attached to nuclear structures might have control functions in genome regulation.

KEYWORDS:
Flavanols, flavonols, DMACA reagent, histology, nuclei.

INTRODUCTION

Both epicatechin and catechin are basic units of oligomeric flavanols or proanthocyanidins. In tree species flavanols were found to have a number of functions, especially protection against climatic stress or biotic aggressors [1] as well as in modification of growth processes and enzymatic activities [2].

In regard to a broad spectrum of medical and pharmacological aspects the role of plant flavonoids as highly effective antioxidants and radical scavengers is well documented [3]. Nuclei are exposed to damaging oxygen radicals [4], and recently the flavonoid cyanidin was found to form a protective complex with DNA [5]. Bond formation of antioxidative flavanols with nuclear proteins might, therefore, have a function in biological systems. Some flavones, such as luteolin, morin and myricetin, inhibit nuclear helicase RepA [6].

Catechins and proanthocyanidins are ubiquitous in plant foods such as fruits and vegetables. Human blood contains after ingestion of black tea up to 0.55 µmol/L total catechins [7]. Rats were found to absorb catechin and quercetin [8] and in the intestinal lumen of rats catechin was still present in its native form [9].

Recently, it was reported that flavanols were capable of associating with the nuclei of the herbaceous *Allium sepa* and the forest tree *Tsuga canadensis* [10]. This work was extended to a larger group of tree species [11].

Polyphenols of plant food, especially vegetables, fruits, juices, teas and wines, are considered to be capable to fulfill essential physiological roles in nutrition [12, 13]. However, information on mammalian cells in relation to absorption and distribution of plant polyphenolic compounds is very limited. In the present study, using DMACA reagent, Naturstoff reagent and light microscopy, first direct evidence is given that in different bovine cells the nuclei were found to bind exclusively or preferentially with added flavanols and flavonols.

MATERIALS AND METHODS

Solution of flavanols and flavonols

Epigallocatechin-gallate (Sigma, Taufkirchen, Germany) was dissolved in a 0.1 M phosphate buffer to get a saturated solution. Rutin (quercetin-3-rutinoside) and isoquercitrin (quercetin - glucoside, both flavonols from Roth, Karlsruhe, Germany) were dissolved in aqua dest. at a concentration of 1 mM each).

Dry grape seeds (*Vitis vinifera* L.) were ground to a fine powder with a ball mill. 10 mg of the powder was extracted in 2 ml water for 2 h on a stirring water bath at 70 °C. The watery phase was separated from the insoluble material by short centrifugation at 500 g and used immediately for incubation. Due to strong browning of the extracts fresh solutions should be prepared for each ex-

periment. Grape seeds contain in addition to the monomeric flavanols (catechin and epicatechin), more than 20 different catechin-based oligomeric proanthocyanidins [14].

Tissue sections

Different methods (A-D) were used to obtain an optimal blue staining reaction of nuclei after incubation in the flavanol solutions using DMACA reagent.

A. Frozen fresh sections of tissues, about 10 µm thick, were immersed directly into a flavanol solution for at least 2- 3 h.

B. Formalin fixed tissues were embedded in paraffin, sectioned at 5 µm and then treated as in A. In the case of spleen some sections were additionally stained with Naturstoff reagent A (Fig. 1: 11, 12).

C. Treatment as in B, however, the paraffin was removed prior to staining by immersing the sections for 2 min in xylene, followed by incubation in absolute, 80 %, and 60 % ethanol for 2 min each. Then, the slides were incubated in a flavanol solution.

D. Treatment as in C, however, the tissues were finally mounted in Eukitt.

Histochemistry

Fresh tissue sections (A) were mounted on microtome slides and then incubated in aqueous saturated solutions of epigallocatechin-gallate or extracts from grape vine seeds for at least 3 h.

In the case of paraffin sections (B-D), a small circular tissue of about 5 mm in diameter was covered for at least 3 h with a drop of the flavanol solution. As the watery solutions did not easily enter into the paraffin layers 0.03 ml of ethanol was added. Before visualizing the flavanols the remaining droplet was removed with a filter paper and the tissue was additionally treated with 2 - 3 drops of water to remove the non-bound flavanols. Paraffin sections of spleen and liver were treated with crude watery extracts from grape seeds in the same way.

For visualizing epigallocatechin-gallate and flavanols of grape vine seeds the selective DMACA reagent (1% p-dimethylaminocinammaldehyde, Sigma-Aldrich, Fluka, Taufkirchen Germany, dissolved in 1.5 N sulfuric acid) was used [1, 15, 16]. The reagent was applied for 10 - 20 min and then the tissue was washed repeatedly to remove the excess of the reagent. All flavanols, irrespective of the degree of polymerization, stain with the DMACA reagent.

In addition to the flavanols, two flavonols were used in the same way to incubate tissues of liver and spleen, namely rutin (quercetin-3-rutinoside) and isoquercitrin (quercetin-3-glucoside). Both flavonols were purchased from Roth (Karlsruhe, Germany). They were visualized with Naturstoff reagent A (diphenylboric acid 2-aminoethylester, 1 % in methanol, purchased from Roth, Karlsruhe Germany). For fluorescence observations a Zeiss microscope was used, fitted with a mercury vapor lamp, excitation filter BP 365, barrier filter LP 435.

Tissue sections, which were not incubated with flavanols or flavonols were used as controls. All experiments were performed with 3 different sections each of brain, intestine, kidney, liver and spleen; per section 3 to 5 different replicates were tested.

RESULTS

Comparison of the different tissue preparations

Fresh tissues (method A, see Materials and methods) allowed a prominent DMACA staining reaction of the nuclei (Fig. 1: 1,4,7,8), whereas control tissues showed no blue nuclei (not presented in Fig. 1). However, depending on the tissue and thickness of the slides some diffuseness around the nuclei was sometimes recognizable. Embedding in paraffin (method B) readily gave a satisfactory blue colouration of the nuclei (Fig. 1: 6, 9-12). Deparaffinized slides (method C) yielded a staining quality similar to the method B (not shown in the present publication). Mounting of the slides in Eukitt (method D) displayed at lower magnifications good brightness of the blue coloured nuclei (Fig. 1: 2,3,5). However, at higher magnifications (x 1000) a slight diffuseness could be occasionally observed due to mounting medium.

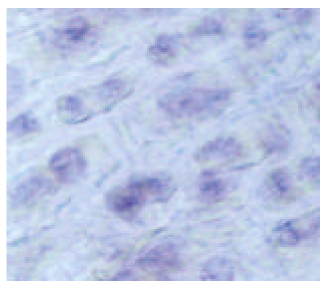
Comparing the 4 different methods it is evident that little differences exist in DMACA staining quality. It appeared that the type of tissue itself was of major importance than the staining method. This result is important since it became evident that the methods A - D apparently allowed a similar diffusion of flavanols towards the nuclei.

The method B turned out to be appropriate for recognition of flavonols (rutin and isoquercitrin) on the basis of Naturstoff reagent A using spleen tissue.

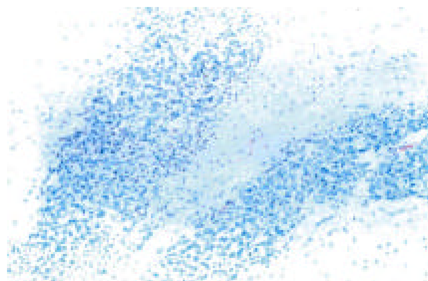
Brain

Nuclei of pia mater were stained by method A. The bluish-violet colouration of the nuclei caused by DMACA is somewhat diffuse, perhaps due to a poor diffusion of both EGCG and colouring reagent through viscous lipoid layers (Fig. 1: 1). Tissue from cortex cerebri was sectioned by microtome, and the paraffin sections embedded in Eukitt (method D) gave a quite prominent staining of the nuclei (Fig.1: 2,3). In Figure 1: 2 there were two distinct zones with high densities of the nuclei, however interrupted by sectors indicating a very low density of nuclei. At a higher magnification the nuclei were more prominent (Fig. 1: 3).

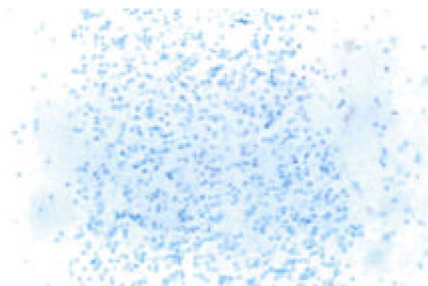
FIGURE 1 - Affinity for flavanols and flavones of nuclei from bovine tissues.



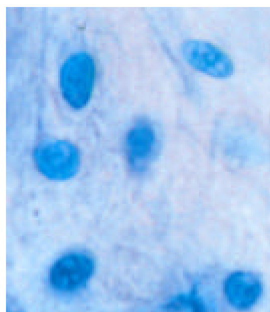
1. Brain, pia mater (EGCG, method A, size of small nuclei 6 µm)



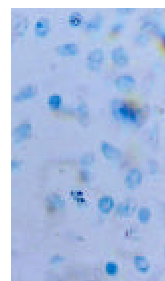
2. Cortex cerebri (method D, EGCG, size of the nuclei 4 µm)



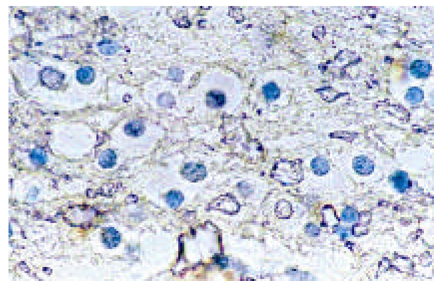
3. Cortex cerebri, magnification of Fig. 1: 2 (method D, EGCG, size of the nuclei 4 µm)



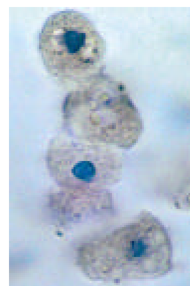
4. Small intestine (method A, EGCG, size of globular nuclei 5-6µm)



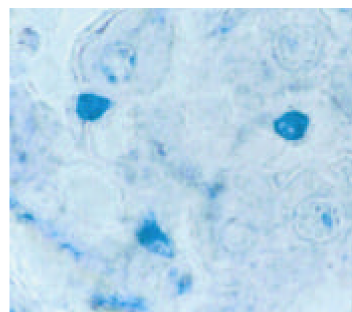
5. Kidney (method D, EGCG, size of the nuclei 4 µm)



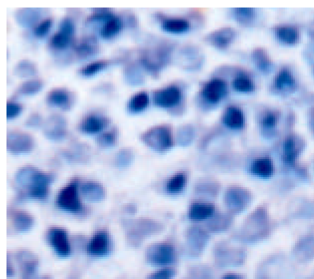
6. Liver (method B, EGCG, size of the nuclei 6 µm)



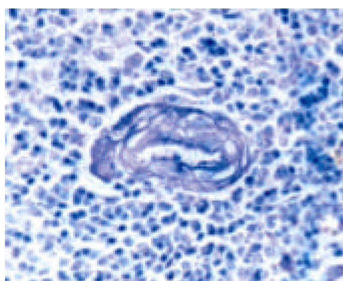
7. Liver (method A, EGCG, size of the nuclei 6 µm)



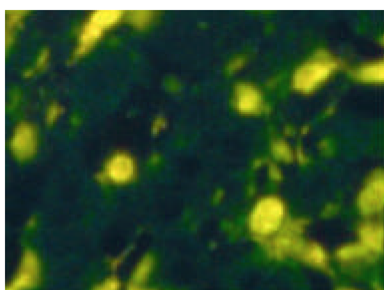
8. Liver (method A, grape vine seed, size of the nuclei 6 µm)



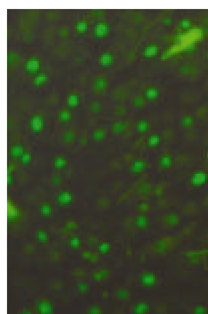
9. Spleen (method B, grape vine seed, size of the nuclei 5 - 6 µm)



10. Spleen (method B, grape vine seed, size of the nuclei 5 - 6 µm)



11. Spleen (method B, isoquercitrin, size of globular nuclei 4 - 6µm)



12. Spleen (method B, rutin, size of the nuclei 5 - 6 µm)

Small intestine

Sections of small intestine were made from fresh tissues (method A). The nuclei were densely arranged and showed a high affinity for EGCG (Fig.1: 4). The background of the microphotograph showed blue coloured streaks, which could not be removed with water.

Kidney

Slides of kidney were treated as described in method D. The nuclei showed a positive reaction for EGCG and were clearly visible with DMACA (Fig.1: 5). Some nuclei were found to have small abnormally condensed particles.

Liver

Distinct liver sections, when stained with DMACA, were often characterized by brown structures along the cellular periphery (Fig.1: 6) or by a diffuse browning of the entire cell (Fig. 1: 7). However, the nuclei appeared to be quite concrete in shape and deep blue coloured. Methods B or A had no influence on the browning reaction (Fig. 1: 6, 7).

A further sector of liver treated with grape vine seed extract showed a quite significant binding potential of the nuclei for the large group of water-soluble flavanols (Fig.1: 8. DMACA).

Spleen

Paraffin sections from the spleen were treated with the extract from grape vine seeds and then stained with DMACA (Fig. 1: 9, method B). The nuclei were found to occur in great density and stain comparatively dark blue. Some blue diffuseness around several nuclei is due to underlying nuclei which are less precisely focused. In addition to the nuclei other structures were associated with the flavanols from grape vine (Fig.1: 10, method B, DMACA).

Spleen nuclei that have taken in isoquercitrin or rutin can be stained with Naturstoff reagent A yielding a bright yellow (Fig.1: 11) resp. green fluorescence (Fig.1: 12). Thus, in addition to the grape vine flavanols also flavonols were found to bind to the nuclei.

DISCUSSION

The results obtained showed that the nuclei of different bovine tissues are a strong sink for the added epigallocatechin-gallate and grape vine flavanols. A prime candidate for nuclear flavanol association would be proteins [17, 18]. Flavanols, which are present in nuclei of a number of tree species *in vivo* [11], may serve as brigding compounds between nuclear structures. This has relevance as a steady remodeling of nucleosomal structures, which is typical for mitotic cells [19]. Another aspect might be that monomeric flavanols and oligomeric proanthocyanidins participate in the antioxidant and antiradical network [20].

In the current study, the entire water-soluble flavanol group of grape seed extract was found to be added to the tissues of the liver and spleen, significant amounts of which were associated with their nuclei. This result is of significance when considering that grape seed polyphenols were found to be cytotoxic against cancer cells but not for healthy ones [21], or reduce endothelin-1 synthesis thus reducing the risk of coronary heart disease [22].

To address a crucial point, the non-treated bovine nuclei did not respond to flavanols as do plant nuclei of a number of tree species. Nuclei of *Tsuga canadensis* are naturally loaded with flavanols to such an extent that additional imbibition of flavanol solutions hardly caused a more intense blue colouration [10, 11]. However, nuclei of the herbaceous *Allium cepa* were a priori devoid of flavanols [10].

As possible limiting factors for a lack of flavanols in bovine nuclei may be hypothetically adressed:

- some differences between plants and animals in structure and function of the nuclear envelope [23]
- a strongly limited number of target sites for flavanols in bovine nuclei
- metabolic modifications of flavanols before entering the bovine nuclei
- the defence system of mammals against bacteria, fungi and viruses is not based as essentially on flavanols as in many flavanol-rich tree species [1]. Alternatively, other flavonoids, as shown with two yellow resp. green coloured flavonols applied to spleen tissue, might be candidates to associate with bovine nuclei.

Concerning yellow fluorescing flavonoids associated with nuclei, only one study has been reported so far for plants [24]. In any case, bioavailability and the metabolic fate of foodstuff flavanols and flavonols in mammals is altogether far from being well understood and warrants further investigation. There is a vast complexity of interactions, which deserves much attention in future research [8].

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PRODUCTION AND EVALUATION OF *Pleurotus ostreatus* MUSHROOM CULTIVATED ON SOME FOOD PROCESSING WASTES

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SUMMARY

Cultivation of *Pleurotus ostreatus* NRRL-0366 on solid cabbage waste from protein extraction process of cabbage leaves was carried out. Mixtures of rice straw with cabbage waste at different ratios were used as substrate for the production of the mushroom fruit bodies. When comparing the biological efficiency of mushroom production, the highest yield (19.65%) of fruiting bodies was obtained using a 1:1 mixture of cabbage waste and rice straw, followed by 11.97% at o.5:1 and 7.09% at 2:1. The lowest yield (3.64%) was achieved with the mixture 3:1. Cultivated mushrooms were found to be good sources of carbohydrates, proteins and fats with contents varying in the ranges 42.24-48.08%, 20.59-26.84% and 4.36-6.40%, respectively. Differences were observed in the amounts of the amino acids. Lysine, arginine, threonine and phenylalanine were the essential amino acids found in all mushroom samples in high concentration. Compared with vegetables, mushrooms proved to be also a good source of many minerals, e.g. K, Na, Zn, Cu, Fe, Ca and Mg. GLC analysis of the fatty acids showed that unsaturated C:16 and C:18 fatty acids accounted for a major fraction. The compost of mushrooms is considered as a highly nutritious fodder for poultry and animals.

KEYWORDS: Mushrooms, solid state fermentation, nutritional value; food processing waste, agricultural waste.

INTRODUCTION

Malnutrition among the populations living in need is still a big problem and forces to search for new food resources. In Egypt, more than 30 million tons of agricultural residues are yearly accumulated. To overcome the pollution of residues from juice and food industries, it was thought as worthwhile to use these semisolid wastes for cultivation of fungal fruit bodies. Mushroom production is one of the few commercial large-scale applications of microbial technology for bioconversion of agricultural and forestry waste materials to valuable foods. Lignocelulosic materials are the main substrates for mushroom production. lt is well known that mushrooms have a high nutritive value, compared to some other vegetables, in protein [1], carbohydrates [2, 3], fat and fiber content [4-6]. Mushroom, especially *Pleurotus*, have the ability to grow on a wide range of unfermented plant wastes. Low laboratory cost, low initial investment and less damage of the fruit bodies by diseases and pests [7] encouraged the authors to develop new techniques for growing *Pleurotus* in large quantities, permitting not only internal consumption, but also exportation.

The purpose of this work was to study the production of edible mushroom by solid state fermentation (SSF), using Egyptian agricultural wastes (cabbage leave wastes and rice straw). Also, the chemical composition of the produced fruiting bodies and the upgraded wastes left were chemically analyzed.

MATERIALS AND METHODS

Organism

Pleurotus ostreatus NRRL-0366, obtained from Agricultural Research Service (Peoria, IL), was maintained in large tubes containing agar potato dextrose [8] incubated at 28-30 °C for 1 week, and stored at 4 °C for one month.

Material

Samples of cabbage leaves were collected from Egyptian local markets during winter season. Cabbage waste (CW) was obtained after extraction of the cabbage leaf protein as described by Goel et al. [9].

Media and Cultivation

Pleurotus species were grown in 250 ml flasks containing 100 g of wheat grain, autoclaved at 121 °C for 1 h, cooled, and then inoculated with cm of mycelium (containing 10 mg dry wt). The mycelia were 1cm incubated at 28 °C for 20-25 days. The medium used for all fermentation experiments, unless otherwise stated, contained the following reagents: 200 g wet cabbage waste, 200 g small pieces of rice straw, 5g $CaCO_3$, and 200 ml distilled water [10]. The cultured-spawn mixture was placed in polyethylene bags containing freshly sterilized media. The bags were closed and incubated for 15 days at 25-28 °C. The bags were opened, perforated along the upper sides, and humidified daily by spraying with water. Mushroom pinheads appeared on all sides after 5 - 7 days. Young mushrooms were harvested from each bag. A second crop appeared after an additional interval of 7 - 10 days. The two crops were collected, lyophilized and weighed. The yields on basis of the total quantity of substrates were compared and the biological efficiencies calculated as described by Gujral et al. [11].

Analytical Methods

The moisture content and ash were determined according to the methods of. [7, 11]. The dry weight was determined gravimetrically and crude protein by Kjeldahl method as N × 6.25 [12], while its true protein was estimated by the method of [13], using bovine serum albumin as a standard. The amino acid composition of fruit body hydrolyzates (acid-hydrolysed with 6N HCl at 110 °C for 22 h [14] was determined with an HPLC Amino Acid Analyzer Eppendorf LC 3000. Lipid content was determined after extraction of the dried fruit bodies with a cold 1:1 mixture of methanol and chloroform [15] and the fatty acid methyl esters were determined with a Hewlett Packard GC (HP 6980 series). The method described by [16] was used to estimate the total carbohydrates of the fruit body hydrolysates [17]. Energy values (Kcal per 100 g dry wt. = 2.62 x (%N x 6.25) + 8.37 x % fat + 4.2 x % carbohydrates) were calculated as mentioned by [18]. Minerals (Zn, Cu, Fe, Na, Mn, Mg and K) in ashed samples were measured with an atomic absorption spectrophotometer [19].

RESULTS AND DISCUSSION

The production of edible mushrooms, using a variety of agricultural and forestry residues as substrates has gained prominence in the last few years. Besides being a delicacy, mushrooms are also an important source of food proteins for human consumption [11, 20].

The four crops produced by cultivated *P. ostreatus* NRRL-0366, on different substrates, were collected, dried and weighed (Fig 1). Table 1 shows the effect of different types of substrates at different ratios using cabbage wastes supplemented with small pieces of rice straw as described in the methods section. The maximum biological efficiency value (19.65%) was obtained when using cabbage wastes and rice straw at 1: 1 ratio (II) followed by I, 11.97% at 0.5:1 ratio and III, 7.09% at 2:1 ratio. The 3:1 ratio (IV) showed the lowest value (3.60%).

FIGURE 1 - Fruit bodies of *P. ostreatus* on cabbage waste and rice straw.

TABLE 1 - Average amounts of fresh fruit bodies of *P. ostreatus* and their biological efficiency on different substrates.

Substrates	Yield of fresh fruit bodies (g/kg dry substrate)		Total yield (g/kg substrate)	Moisture (%)	Biological Efficiency (%)
	1st flush	2nd flush			
I	366.20	111.73	477.93	74.96	11.97
II	602.50	293.60	896.10	78.10	19.65
III	448.10	49.60	497.73	85.75	7.09
IV	219.25	104.85	324.10	82.61	3.64

Cabbage waste + rice straw ratios: I (0.5:1 w/w); II (1:1 w/w), III (2:1 w/w) and IV (3:1 w/w).
These values are means of three experiments.
Biological Efficiency (%) = g dry fruit bodies per 100 g dry substrates.

TABLE 2 - Chemical composition of *P. ostreatus* fruit bodies cultivated on different substrates.

Substrates	Crude protein (%)	True protein (%)	Total carbohydrates (%)	Crude fat (%)	Ash	Energy value (kcal/100 g)
I	29.91	24.39	46.84	6.20	6.40	327
II	30.66	21.52	48.08	6.40	6.80	336
III	38.01	26.84	42.24	5.00	9.20	319
IV	35.87	20.59	43.92	4.36	10.00	315

TABLE 3 - Amino acid composition (mg g^{-1} protein) of the fruiting bodies of *P. ostreatus* cultivated on different substrates.

Amino acid	I	II	III	IV	FAO/WHO (1991) requirement pattern
Aspartic acid	28.80	30.00	41.55	23.40	
Threonine	14.12	14.80	24.00	10.31	34
Serine	16.41	17.90	25.85	2.16	
Glutamie acid	54.45	66.90	129.27	45.70	
Proline	25.70	20.70	33.20	15.37	
Glycine	16.65	16.10	23.27	11.06	
Alanine	20.72	22.90	34.13	16.97	
Cystine	0.69	0.40	1.46	0.50	
Valine	11.23	11.40	20.92	8.90	35
Methionine	1.73 (2.42)	2.10 (2.50)	3.16 (4.62)	1.80 (2.3)	25[a]
Isoleucine	8.70	8.70	15.48	6.86	28
Leucine	15.59	16.50	22.63	10.35	66
Tyrosine	8.57	9.90	17.42	8.30	
Phenylalanine	12.75 (21.3)	12.90 (22.8)	22.72 (40.14)	11.90 (20.2)	63[b]
Histidine	17.60	17.60	18.91	4.90	
Lysine	11.31	14.50	24.85	10.80	58
Arginine	20.63	24.80	40.96	17.20	
Total essential amino acids	75.43	80.90	133.76	60.92	

Values are the averages of two determinations.
[a] Cystine + Methionine
[b] Tyrosine + Phenylalanine.

Data shown in Table 2 illustrate the chemical analysis of *P. ostreatus* fruit bodies. The crude protein contents in the fruit bodies cultivated on cabbage plus rice straw at different ratios (0.5:1, 1:1, 2:1, 3:1) were 29.91, 30.66, 38.01 and 35.87%, respectively. The highest value (38.01 %) was obtained when double the amount of cabbage waste was used in the mixture with rice straw. *Gujral et al.* [11] observed a significant variation in protein content (26.60-35.50%) when *P. sajor. caju* was cultivated on different substrates. Nearly identical variations in protein content of six *Pleurotus* spp. cultivated on wet wheat straw during winter (26.97-31.96%) were recorded by the investigators [6].

The changes in total carbohydrate content of the fruit bodies varied within narrow limits (42.24-48.08%; Table 2). Proteins and carbohydrates represent the major constituents of the fruit bodies using the different media. These results were in accordance with those mentioned by [7, 10] who found when using different species of *Pleurotus*, total carbohydrate contents of 57.4 –81.8%. The crude fat content of the fruit bodies of *Pleurotus ostreatus* cultivated on different substrates ranged from 4.36—6.4% on dry wt. basis and were similar to those mentioned by Bano and Rajarathnam [7] or Jwanny et al. [10]. They stated that the fat content in different species of *Pleurotus* ranged from 1.08-9.40% on dry wt. basis [7, 10]. On the average, *Pleurotus* species contain 2.85% fat. Also nearly similar calorific values ranging from 315 to 336 Kcal per 100 g of dry mushrooms for the four different substrates were found. Similar results were recorded by many investigators [7, 18, 21].

As it has been reported that the proportions of the amino acids depend on the culture medium [22, 23], the amino acid profiles of the mushrooms samples were determined and presented in Table 3. The amino acids analysed represent both the free and bound amino acids. It can be seen that the essential amino acids (threonine, methionine, leucine, phenylalanine and lysine) are present in high quantities except tryptophan, which was not measured. The mushroom fruiting bodies of sample III contained all the amino acids in higher amounts. These results agree with those found for *P. ostreatus* "Florida" F6 fruit bodies which contained about 17.2 g% dry wt. of total amino acids, of which the essential amino acids were about 14.54% [24]. The fruit bodies of *P. ostreatus* had lower levels of aspartic and glutamic acid than the fruiting bodies previously examined [25]. It can be concluded that mushroom is a good source of protein containing lysine, arginine, and threonine in high concentrations.

It is not surprising to find that the relative amounts of amino acids in the mushroom samples were identical to those grown on other media under different conditions.

TABLE 4 - Effect of substrate type on the mineral content of *P. ostreatus* fruit bodies.

Substrates	Ash	Minerals (mg/100 g dry fruit bodies)							
	(%)	Zn	Cu	Fe	Na	Mn	Mg	K	Ca
I	6.4	7.0	11.85	209.85	654.0	1.85	165.80	654.50	19.00
II	6.8	8.9	6.00	82.45	503.8	2.05	165.40	2000	11.86
III	9.2	7.2	10.20	130.30	610.0	3.50	154.40	2020	10.86
IV	10.0	7.7	6.40	42.85	433.4	1.60	136.00	631.50	13.64

TABLE 5 - Fatty acid composition of fruit bodies of *P. ostreatus* cultivated on different substrates.

Lipid -acid	**P. ostreatus**			
	I	II	III	IV
C 14:0	-	2.89	3.30	3.50
C 15:0	-	-	4.00	-
C 16:0	7.66	3.36	3.04	2.90
C 16:1	10.68	13.10	13.12	11.63
C 17:0	-	-	-	4.53
C 18:0	21.22	13.84	7.48	8.60
C 18:1	46.92	59.70	61.72	54.60
C 18:2	7.25	4.25	-	-
C 20:0	6.27	2.86	7.34	14.24
Saturated	35.15	22.95	25.16	33.77
Unsaturated	64.85	77.05	74.84	66.23

TABLE 6 - Effect of mushroom fermentation on the contents of components of cabbage/rice straw compost media.

Components (% dry wt.)	*P. ostreatus*							
	Control	Fermented	Control	Fermented	Control	Fermented	Control	Fermented
	I	I	II	II	III	III	IV	IV
Crude protein	2.28	5.00	3.50	6.50	5.25	9.63	5.20	6.00
Crude lipid	9.38	4.84	10.76	10.58	11.14	7.50	11.00	5.02
Crude ash	6.50	9.00	3.60	7.60	5.00	8.70	14.70	16.10

Compared with vegetables, mushrooms also proved to be good sources of many mineral elements. Table 4 shows that the fruit bodies of *P. ostreatus* generally contain about 6.4-10.0% ash representing the minerals. The effects of the different substrate types on the mineral contents of *P. ostreatus* fruit bodies determined in ash are shown in Table 4. Potassium, sodium and magnesium were found to be the main constituents. Zinc, copper calcium and manganese are present in low concentrations in all the media. Iron is present in high amounts, especially when using medium I, but iron is present in only low concentration when using medium IV. These results are important, since Bano and Rajarathanam [7] indicated that iron, present in *Pleurotus* species, is available and utilized in the presence of other enhancing factors, such as proteins and ascorbic acid.

The fatty acid composition of the purified and esterified lipids in *P. ostreatus* fruit bodies, cultivated on different substrates, are shown in Table 5. Fatty acids with both even and odd numbered carbon atoms between C_{14} and C_{18} could be observed. GLC analysis of the fattv acids has revealed that unsaturated C:16 and C:18 fatty acid are the major fraction. C18:1 was found to be the main component comprising 64.85, 77.05, 74.84 and 66.23% of the total acids in fruit bodies cultivated in media I, II, III and IV, respectively.

Results in Table 5 were similar to some extent to those of Kajuno and Stancher [26, 27] revealing that the major fraction of fatty acids of *P. ostreatus* mushrooms consisted of 18:0, 18:1 and 18:2. It can be concluded that *P. ostreatus* fruit bodies cultivated on different substrates show relatively high concentrations of nutritionally valuable unsaturated fatty acids, especially the important essential dietary constituents oleic and linoleic acid are as reducing agents in chronic heart diseases.

Table 6 shows the analytical results of the remaining compost, which was oven-dried at 60 °C after mushroom cultivation on cabbage waste/ rice straw mixtures and harvesting of the fruit bodies. The crude protein content was significantly increased in the fermented samples of media I to III, while the crude lipid content was substantially decreased after fermentation in all media samples. These data suggest that the compost could be used as a nutritious animal feed.

CONCLUSION

Mushroom cultivated on cabbage waste mixed with rice straw could be considered as a good source of protein containing reasonable amounts of essential amino acids and unsaturated fatty acids. Compared with vegetables, the mushrooms also proved to be good sources of many mineral elements, e.g. K, Zn, Fe, Na and Cu in considerable amounts. The remaining compost could be used as animal fodder.

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AFS Book Reviews - Bücherschau

Auditing in the food industry – From safety and quality to environmental and other audits

Mike Dillon and Chris Griffith (Eds)

Woodhead Publishing in Food Science and Technology

217 pages, tables; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd, Abington Hall, Abington, Cambridge, CB1 6AH, UK, **July 2001**; **ISBN 1-85573-450-8**; Hardcover **£ 115.00/€190.00** (plus p&p).

This book represents an essential guide to the range of standards and auditing skills required for the modern food industry. The consumers are interested in safety and quality of food products and the way in which they are produced. Therefore, the food industry has developed new ways of assuring appropriate standards for its products and methods of production such as TQM or HACCP.

In Part 1 an introductory chapter reviews developments in standards affecting the food industry followed by several chapters concerning on how retailers audit their suppliers and how governments have moved from a traditional inspection role to one of "regulatory verification" with its emphasis on auditing the robustness of a business´s own systems for managing safety and quality.

Part 2 elucidates the important key aspects of safety and quality. At first, the ways retailers assess supplier HACCP systems is reviewed. Then TQM systems that provide a context for a discussion of auditing techniques for HACCP-based quality systems are discussed. Finally, the standards governing the analytical methods practised in safety and quality control are considered.

Part 3 of this book describes the newer standards that are becoming increasingly important in the food industry. The reader will find chapters on benchmarking an organisation against others as a way of improving performance, auditing the impact of food processing operations on the environment and organic food processing.

This book is a valuable guide to the range of standards facing the food industry, the ways it can audit, and thus improve the quality of its performance and is recommended for scientists and practitioners in academia, industry and public authorities.

FROM THE CONTENTS

Introduction; Part 1 – The auditing process (Food standards and auditing; What auditors look for: a retailer´s perspective;Regulatory verification of safety and quality control systems in the food industry); Part 2 – Safety and quality (Assessing supplöier HACCP systems: a retaile s perspective; TQM systems; Auditing HACCP-based quality systems; Laboratories and analytical methods: quality control); Part 3 – Other types of audit (Benchmarking; Environmental audits and life cycle assessment; Auditing organic food processors); Index.

Instrumentation and sensors for the food industry

Erika Kress-Rogers and Christopher J. B. Brimelow

Woodhead Publishing in Food Science and Technology

2nd edition; **836** pages, numerous tables and figures; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd, Abington Hall, Abington, Cambridge, CB1 6AH, UK, **Dec. 2001**; **ISBN 1-85573-560-1**; Hardcover **£ 175.00/€ 270.00** plus (p&p).

The first edition of this handbook established itself as the standard reference on instrumentation for measuring the quality of food. Because of the rapid change in this area this second edition is a substantially new and longer book with 16 new contributors and 8 completely new chapters, as well as major revisions to the already existing chapters of the first edition.

After the two fundamental introductory chapters "Instrumentation for food quality assurance" and "Instrumental measurements and sensory parameters" Part 1 of this handbook covers the in-line measurement of food processing operations by a range of techniques, such as measurement of colour, food composition, pressure, tempara-ture, levels, flow and viscosity. Part 2 deals with instrumental techniques in quality control laboratories including measurement of rheological properties, texture, water and microbiological activity. Part 3 consists of five chapters introducing to ther increasingly widespread use of electronical noses and tongues, chemosensors, biosensors, immunosensors and DNA probes.

This book is recommended to all scientists and practitioners working in industry, academia or public authorities in the field of food science.

Lockhart & Wiseman`s crop husbandry including grassland

H. J. S. Finch, A. M. Samuel and G. P. F. Lane

Woodhead Publishing in Food Science and Technology

8th edition; **510** pages, numerous tables and figures; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd,

Abington Hall, Abington, Cambridge, CB1 6AH, UK, **May 2002**; **ISBN 1-85573-549-0**; Softcover **£ 35.00/€ 55.00** plus (p&p).

This 8th edition is completely and comprehensively revised, but also expanded and confirms its reputation as a classic. This book, first published in 1966, has established itself as the standard crop husbandry, especially for students and practitioners.

Part 1 – Conditions for Crop Growth – deals with plant structure and growth, soil analysis and management, the use of fertilizers and manures, weeds, pests and diseases of farm crops. In a new chapter the influence of climate and weather conditions on crop growth is described. Part 2 explains general aspects of crop husbandry, such as cropping techniques including new chapters on the important areas of integrated crop management and organic crop husbandry as well as a discussion of seed selection and production. Part 3 is an application section examining how these general techniques are used with particular crops, such as cereals, root crops, fresh harvested crops, forage crops and combinable break crops. In the final Part 4 the use of grassland is considered in chapters on characteristics of grassland and the important species, establishing and improving grassland, grazing, and conservation of winter feed. The book closes with Appendices containing chapters on soil texture assessment in the field, nomenclatures of crops and weeds, insect pesticides, crop diseases and seeds, metrication, agricultural land classification in England and Wales, weed control and a Subject Index.

This handbook is employed to all interested readers as a valuable and practical reference guide for the farming industry.

Food chemical safety – Volume 2: Additives

David H. Wilson (Editor)

with contributions of *David Watson, W. Flowerdew, Patricia Curtis, David Tennant, Roger Wood, Christopher Blake, Mark A. Kantor, F. Jack Francis, Kenneth R. Schrankel, Gert von Rymon Lipinski, Brian Whitehouse, Kamila Míková*

308 pages, numerous tables and 14 figures; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd, Abington Hall, Abington, Cambridge, CB1 6AH, UK, **Febr. 2002**; **ISBN 1-85573-563-6**; hardback **£ 135.00/€ 210.00** plus (p&p).

Volume 1: Contaminants of this new two-volume treatment of food chemical safety (Analytical methods; particular contaminants; international regulation of chemical food contaminants in the EU and US) was already published in 2001. Volume 2 covering analysis and control of the use of additives in food processing and regulation of additives in the EU and US is now available.

In the meanwhile the use of additives in foods is widespread, but still controversial in the view of consumers. Especially reservation about the safety of additives used to enhance quality parameters of food products, such as taste, colour, texture and shelf-life, has been expressed. These facts have increased the pressure on food producers to demonstrate the safe use of additives. Therefore, the editor with a team of co-working experts elucidates the regulatory context and the methods used to analyse, assess and control their use in food processing. In the first part of Volume 2 an overview of EU and US regulations is presented. On the basis of Part 1 risk analysis in assessing the impact of additives on consumer health, quality control of the analytical methods and also new, more rapid and targeted methods for detection and measurement of additives are explained. Integrated is an important review of adverse reactions to additives covering such issues as monitoring, reporting trends and evidence concerning the most important additives. In the last part some of the key groups of additives (colorants, flavouring and texturing agents, sweeteners, antioxidants) are presented more detailed.

Handbook of herbs and spices

K. V. Peter (Editor)

with contributions of *K. V. Peter, Maria Clay and Martin Muggeridge, Rehka S. Singhal, C. K. George, Mensure Özgüven, Sushil Kumar, P. N. Ravindran and Johny A. Kallupurackal, T. G. Berke and S. C. Shieh, V. S. Korinkanthimath, K. J. Madhusoodanan and Y. Saideswara Rao, J. Thomas and P. P. Duethi, N. Nurdjannah and Nurliani Bermawie, Gholamreza Amin, Rajendra Gupta, U. B. Pandey, P. A. Valsala, V. K. Rajuand M. Reni, S. N. Potty and V. Krishna Kumar, B. Krishnamoorthy and J. Rema, K. E. Lawande, P. Pushpangadan and S. P. Singh, Nedyalka V. Yanishlieva-Maslarova, Arturo Velasco-Negueruela, Y. Saideswara Rao and K. Mary Mathew* and *B. Sasikumar.*

319 pages, numerous tables and figures; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd, Abington Hall, Abington, Cambridge, CB1 6AH, UK, **Dec. 2001**; **ISBN 1-85573-562-8**; hardback **£ 150.00/€235.00** plus (p&p).

Herbs and spices are among the most important and versatile ingredients in foods and food processing. Their traditional role is flavouring and colouring foods, but in

the recent years they have increased their importance also as natural preservatives and bacause of their potential health-promoting properties, for example as antioxidants. The editor of this handbook of herbs and spices is a leading authority in the field and was supported by a distinguished international team of contributors to provide manufacturers and scientists engaged in this field with an essential reference guide.

The Introduction section covers definition of spices and herbs, the trade in spices, spice flavours, processing issues, the functional role of spices, and finally the structure of this handbook, additional sources of further information or advice completed with an ISO list of plant species containing the plant parts used as spice. In the following two chapters quality specifications and indices for herbs, spices and spice essential oils are illustrated. In Chapter 4 spices from organic farming are described. In Capters 5 to 26 major spices and herbs from aniseeds, bay leaves, black pepper, chillies and paprika, cardamom, cinnamon, clove, cumin, curry leave, dill, garlic, ginger, kokam and cambodge, marjoram, nutmeg and mace, onion, poppy, rosmary and sage, saffron, tamarind and turmeric are presented covering key issues from definition and classification in each chapter including chemical structure, cultivation, post-harvest processing, uses in food processing, functional properties, regulatory issues, quality indices and methods of analysis.

This Handbook of herbs and spices will be a standard reference and is recommended to all manufacturers using herbs and spices in their products as well as to food scientists, especially to those engaged in flavour research.

Extrusion cooking – Technologies and application

Robin Guy (Editor)

with contributions of Robin Guy, Mian N. Riaz, Jérôme Mottaz, Jay Sellahewa, Charlie Chessari, Mary Ellen Camire, Jean-Marie Bouvier and Massoud Kazemzadeh

206 pages, **50** figures and numerous tables; CRC Press Boca Raton – Boston – New York – Washington D.C., published in Europe by Woodhead Publisihing Ltd, Abington Hall, Abington, Cambridge, CB1 6AH, UK, **June 2001**; **ISBN 1-85573-559-8**; hardback **£ 115.00/€ 190.00** plus (p&p).

The intension of the editor was to present a major new reference guide for improving efficiency and quality in extruded products. Extrusion cooking is ideal for manufacturing food products from snacks and breakfast cereals to baby foods. To maintain quality the multivariate production process requires a careful control. The key parameters influencing quality and their control during manufacturing is described by an international team of experts in this field.

In the first part general influences on quality, such as selection of raw materials, criteria for selecting the right extruder, analysing and optimising thermal performance in extrusion cooking , effective process control, and maintaining nutritional quality in extruded products are described.

In Part II the application of extrusion in particular, already above-mentioned product groups is covered. Each chapter examines the range of extruded products within the group, the specific production issues and future trends.

Taschenatlas der Biotechnologie und Gentechnik

Rolf D. Schmid

340 Seiten, **139** Farbtafeln; WILEY-VCH Verlag GmbH, Weinheim, **2002**; **ISBN 3-527-30865-2**; Softcover **€29.90**.

Die Biotechnologie ist eine der Schlüsseltechnologien des 21. Jahrhunderts und verbindet Wissensgebiete wie allgemeine Biologie, Molekulargenetik, Zellbiologie, Humangenetik, molekulare Medizin, Virologie, Mikrobiologie und Biochemie, Enzymtechnologie, Bioverfahrenstechnik, Kybernetik und in zunehmendem Maße auch Bioinformatik und Systembiologie aufgrund ihrer Interdisziplinarität. Vor diesem Hintergrund ist leicht einzusehen, daß es bisher keine kurzgefaßten Lehrbücher gibt, die das breit gefächerte Spektrum komplett abdecken. Selbst mehrbändige Monographien lassen oft wichtige Teilgebiete wie Tier- und Pflanzenzucht oder Bioinformatik außer acht. Der Autor hat während seiner Studentenzeit und Lehrtätigkeit an der Universität erfahren, daß der Blick auf das „Ganze“ faszinierend und motivierend ist, wenn das Studium die Aufnahme Tausender zunächst zusammenhangloser Einzelheiten erfordert. Daher hat er mit diesem Taschenatlas Biotechnologie/ Gentechnik ein kleines Handbuch geschaffen, das dem Leser quasi als roter Faden dient, um aus dem Labyrinth dieser zwar anspruchsvollen, aber durchaus zugänglichen Wissenschaft herauszufinden. Dem knappen Text sind logisch aufgebaute Farbschemata von Ruth Hammelehe beigefügt, die die Fülle des abgedeckten Stoffes in einen übersichtlichen Rahmen zwängen.

Nach einem kurzen historischen Überblick beginnt das Buch mit der Biotechnologie der Lebensmittel und beschreibt die Herstellung von Alkoholen, Säuren und Aminosäuren, Antibiotika, Enzyme sowie Back- und Futterhefe. Die nächsten Abschnitte beschäftigen sich mit Biotechnologie und Umweltschutz und der medizinischen Biotechnologie (gentechnisch erzeugte Präparate; neue

Erkenntnisse der Erforschung ambryonaler und adulter Stammzellen; Potential rekombinanter Antikörper; Einsatzgebiete für Biosensoren). Ein großer Abschnitt ist dem Thema Landwirtschaft und Biotechnologie gewidmet (moderne Methoden in Tier- und Pflanzenzucht). Nach dem anwendungsorientierten ersten Teil werden im zweiten Teil dieses Taschenatlas wissenschaftliche und technische Grundlagen der Biotechnologie (Bereiche Mikrobiologie und Bioverfahrenstechnik; Molekulargenetische Methoden) kurz und prägnant erläutert. Schließlich rundet der Herausgeber das Buch mit Aktuellen Trends in der Biotechnologie und einem Kapitel über Sicherheit, Ethik und Ökonomie ab. Ein ausführliches alphabetisches Sach- und nach Schwerpunkten dieses Buches geordnetes aktuelles Literaturverzeichnis ist hilfreich für den Leser bei der Erklärung schwieriger Fachbegriffe.

Dieser empfehlenswerte Taschenatlas bietet einen ersten Über- und Einblick in die vielen Arbeitsbereiche der Biotechnologie und wendet sich daher in erster Linie an Studenten der Biologie, Biochemie und Bioverfahrenstechnik.

Handbuch der analytisch-chemischen Aufschlussmethoden

Rudolf Bock

327 Seiten, zahlreiche Abbildungen und Tabellen; WILEY-VCH Verlag GmbH, Weinheim, **2001**; **ISBN 3-527-29791-X**; Gebunden **€109.00**.

Häufig steht man vor der eigentlichen Analyse von Inhaltsstoffen vor dem Problem: Wie schliesse ich das vorliegende Probenmaterial auf und wie bringe ich den nachzuweisenden Inhalsstoff in Lösung. Es gibt eine Unzahl von Möglichkeiten und auch zahlreiche Monographien, die aber meist nur einen Teil der möglichen Aufschlussmethoden beinhalten. Das vorliegende Handbuch unterscheidet sich von diesen monographien durch die Vollständigkeit der Methoden aus den Aufschlussbereichen Lösen und Aufschliessen durch Energiezufuhr; Lösen und Aufschliessen unter Eintritt von chemischen Reaktionen ohne Wertigkeitsänderungen; Oxidierende Verfahren und Reduzierende Verfahren.

Der Autor hat unter Mithilfe zahlreicher Kollegen ein seit Jahrzehnten vergriffenes Buch über Aufschlussmethoden neu bearbeitet und ergänzt. In dem einführenden Kapitel wird zunächst eine Übersicht der wichtigsten Aufschlussmethoden geboten, anschließend werden Ausführung von Aufschlüssen mit Gasen und Flüssigkeiten sowie in der Schmelzeapitel beschrieben und die Behältermaterialien (Glas, Porzellan, Quarzglas, Oxide, Metalle, Graphit u. glasartiger Kohlenstoff, hochpolymere Kunststoffe) diskutiert. Die Einführung wird vervollständigt durch die weiteren kapitel über Fehlerquellen beim Lösen und Aufschliessen; Verluste durch Verspritzen, Verstäuben, Verflüchtigung, Adsorption und Reaktion mit Behältermaterial; Blindwerte; Beseitigung von Schaum sowie Beschleunigung von Aufschlüssen durch Erhitzen mit Mikrowellen, weitere Maßnahmen (Katalysatoren, Ultraschall) und Automatisierung.

Im Hauptteil des Buches werden dann die einzelnen Verfahren (Nass- und Schmelzaufschlüsse, Oxidationsmethoden, Fluorierung, Chlorierung, Sulfurierung, reduzierende Verfahren u.a.) und eine Vielfalt an zu behandelten Analysenproben (Metalle und Legierungen; Nichtmetalle; Silikate; Glassorten; Keramik; Gesteine; verschiedene Erze; Mineralien; Bodenproben; Sedimente; Oxide; Fluoride; Carbide; Nitride; Boride; feuerfeste Materialien; A-schen; Schlacken; u.a.) ausführlich vorgestellt. Besonders zu erwähnen ist das ausführliche Literaturangebot mit über 2000 Zitaten, das eine vertiefte Einarbeitung in spezielle Aufschlussprobleme bzw. -ergebnisse sicherlich erleichtert. Alles in allem, ein gelungenes und empfehlenswertes Handbuch, das dem Analytiker wertvolle Dienste leistet, wenn es um den Nachweis schwer in Lösung zu bringender Matrixbestandteile bzw. –inhaltsstoffe geht.

Vademecum for Vitamin Formulations

Volker Bühler

2nd revised edition, **144** pages, **149** figures, **119** tables, **1** CD-ROM; Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, **2001**; **ISBN 3-8047-1834-5**; Softcover **€39.90**.

Vitamin products are well-known since several decades ago. But there is still much that is obscure in the pharmaceutical technology of vitamins, a class of substances associated with specific problems. Multivitamin products are generally combining such a large number of active substances with entirely different chemical structures and physical properties. Virtually all these vitamins are more or less unstable when formulated and some of them interact to rersult in decomposition. In the literature a lot of publications can be found on the pharmaceutical technology of vitamin formulations.

In this book an overview is given, but combined with stability test results of the author and his team, which were developed in the food products/pharmaceuticals application laboratories of BASF AG, Ludwigshafen, FRG.

On this basis, the 2nd edition aims to make the process of development of vitamin products intelligible and is directed at all experts in the pharmaceuticals, cosmetics and food industries who are engaged in research, development and manufacture of vitamin preparations.

This completely revised edition is supplemented with new formulations of vitamin combinations (e.g. multivitamin syrup, vitamin C + E tablets, Vitamin B complex injectable, multivitamin effervescent tablets, multivitamin tablets with minerals). Also a detailed chapter of multivitamin solutions is added to impart an even better knowledge on the pharmaceutical technology of vitamins.

This recommandable book has the structure of a dictionary and as Introduction an Index of all the vitamin formulations included in this book The handy alphabetical format and many crosslinks between the individual sections of the book guarantee a quick and optimal access to all specific formulations and cross-linked information about auxiliaries in conjunction with the vitamins. Therefore, a CD-ROM (electronic version of the book) is also ncluded.

AFS PRESS RELEASES

INTERNATIONALE FRESENIUS FACHTAGUNG Food Safety and Dietary Risk Assesssment (5.– 6. Dezember 2002, Mainz, Germany)

Der Nitrofen-Skandal hat die Bedeutung der Lebensmittelsicherheit und die Gefährdung durch Pflanzenschutzmittel auf einen vorderen Platz der politischen Tagesordnung gehoben. Mit der Europäischen Lebensmittelbehörde richtet die EU ein neues erweitertes Schnellwarnsystems für Gefahren bei Lebens- und Futtermitteln ein. Wie können Landwirtschaft und Futtermittelhersteller den neuen Anforderungen entsprechen? Welche neuen Verfahren der Risikoabschätzung stehen zur Verfügung? Antworten auf diese Fragen möchte die internationale Fresenius-Fachtagung am 05. und 06. Dezember in Mainz geben.

Auf Einladung der Akademie Fresenius (Dortmund) und der Institut Fresenius Gruppe (Taunusstein) diskutieren internationale Experten aus Wissenschaft, Politik und Praxis, aus Deutschland, Europa und den USA, aktuelle Fragen zu Gefährdungspotentialen, zur Expositionsabschätzung sowie zur Rolle der Europäischen Lebensmittelbehörde bei der Beschreibung und Überwachung von Risiken. Eine begleitende Fachausstellung rundet das Programm der zweitägigen Konferenz ab. Tagungssprache dieser internationalen Konferenz ist englisch. Bei Anmeldung bis zum 16. August 2002 gewährt der Veranstalter 10 Prozent Frühbucher-Rabatt. Das komplette Tagungsprogramm im Netz: www.akademie-fresenius.de

REFERENTEN (Auswahl):

Dr. Ursula Banasiak, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA)

Dr. Bas Drukker, EU-Kommission

Dr. Gunter Fricke, Nestlé Deutschland

Caroline Harris, Novigen Sciences

Dr. Nina Heard, Syngenta Crop Protection

Dr. Jacob van Klaveren, RIKILT Institut

Dr. David Miller, US EPA Environmental Protection Agency

Dr. Alfons Sagenmüller, Bayer CropScience

Termin:	05. - 06. Dezember 2002
Ort:	Atrium Hotel, Mainz
Tagungsgebühr:	1.395,- EUR + MwSt

KONTAKT

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1st INTERNATIONAL CONFERENCE "Quality and risk assessment on agricultural food in the Mediterranean area" (24th – 27th September 2002, Foggia - ITALY)

Under the auspicious of: Istituto Sperimentale per la Cerealicoltura - Sez. Op. di Foggia (Italy), Istituto Superiore della Sanità (Italy), Istituto Zooprofilattico della Puglia e della Basilicata (Italy)

PROGRAM

Tuesday 24th Sept.
REGISTRATION

Wednesday 25th Sept.

SESSION I: UE Politics and Legislation on food safety
SESSION II: Risk assessment
SESSION III: Meat

Thursday 26th Sept.

SESSION IV: Milk and cheese
SESSION V: Wine
SESSION VI: Vegetables
SESSION VII: Fish and cereals

POSTER SESSION

A title and a brief abstract must be send before the 31 th of August to the follow email address:

email: lab.biomol@tiscali.it

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For registration, further information and hotel reservations, please do not hesitate to contact the **Organizing Secretariat:**

G. Spano, L. Benedice, D. Tarantino or
M.G. Minischetti

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