PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF FRUITS AND VEGETABLES WASTE

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Abstract
This study assesses the relationship between physico-chemical and microbiological parameters of fruits and vegetables waste which can be used as raw materials for composting. Two types of food waste were considered in this evaluation: fruits (apple, banana, orange and kiwi peels) and vegetables waste (potatoes peels, cabbage leaf and carrots peels). Several colonies of microorganisms were found on the fruits and vegetables waste. Staphylococci and simple bacilli are the main microorganisms identified on all food waste and were considered for the evaluation in this study. Most of staphylococci colonies were found on potatoes peel (2.34E+06 CFU g^{-1}) and orange peels (2.03E+06 CFU g^{-1}), while large colonies of simple bacilli were found on orange peels (2.14E+06 CFU g^{-1}). Yeasts (2.44E+06 CFU g^{-1}) were identified only on kiwi peels. Also, pH, electrical conductivity (EC), moisture (M) and nitrogen (N) content were determined. Cabbage leaf has the highest moisture (91%), followed by carrot peels (90%) and banana peels (88%), while the lowest moisture was registered for orange peels. The nitrogen content for each food waste sample evaluated is below 1%. Poisson regression analysis was used to assess relationship between microorganisms and physico-chemical indicators. Results showed that all four physico-chemical indicators used as predictors have a significant influence on microorganisms.

Keywords: food waste, moisture, nitrogen content, pH, regression analysis, staphylococci

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1. INTRODUCTION

Oxygen, temperature, particle size, moisture content, pH, C/N ratio are some of the factors that have a highly influence on microorganisms activity (Zhang et al., 2013; Bohacz, 2018). Aeration during composting process supports the aerobic microbial activity and oxygen consumption is an indicator of this activity. According to Bernal et al. (2009), the optimal temperature for the composting process is 40–65 °C. Excessive temperature limits the microbial activity which leads to low grade of organic matter decomposition and also can increase the ignition risk and ammonia emission (Zhang et al., 2013). Microorganisms are growing on particle surface and small particles (20-40 mm) are more rapidly degraded, thus the particle size influences the surface area of compost and porosity (McKinley, 2008).

Moisture content influences the microbial activity during composting process because water is an important medium to transfer nutrients and dissolved gas across the microbial cell membrane, and a 40-60% moisture content is considered as optimum for composting (Gajalakshmi and Abbasi, 2008). It is considered that microbial activity is most efficient at an optimum pH and for the composting process a pH above 7 appears to be preferred by microorganisms (McKinley, 2008). At low values of pH and oxygen, Sundberg et al. (2013) found as the dominant bacteria *Lactobacillales*, while in other studies, optimal pH values for growth of bacteria and fungi are between 6-7.5 and, respectively, 5.5-8 (Amir et al., 2005; Rich et al., 2018). According to Zhang et al. (2013) microbial activity at C/N ratio greater than 35 is significantly influenced since microorganisms must oxidize the excess carbon. For initial mixtures, C/N ratio should be 30-35 in order to ensure the optimal conditions for decomposition and transformation of organic matter by microorganisms. Lower values than

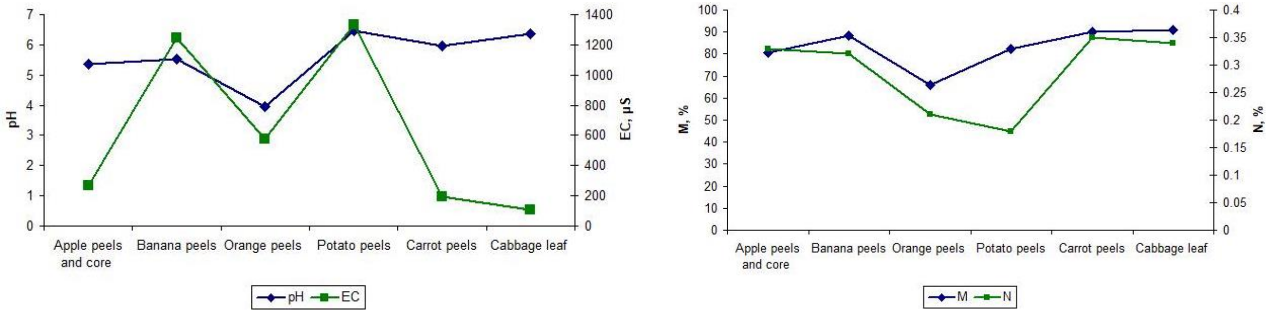
25 of C/N ratio would lead to an increase of the composting time (Sánchez et al., 2017).
In the initial mixture obtained from a mixture of tannery sludge, sawdust, chicken manure and rice bran, before composting (Ahmed et al., 2007) found 5 x 10^6 CFU (colony forming units) for total aerobic counts, 8x10^9 CFU/g for bacilli while also identifying *Salmonella* spp., *Shigella* spp., yeasts and moulds. Bacteria such as *E. coli*, along with cellulolytic, amylolytic and nitrogen fixing species were identified on different substrates (apple peels, banana peels, orange peels and potato peels) by Nasreen and Qazi (2012). For *E. coli*, 24.1±19.5 x 10^2 CFU were found on apple peels and 33.7±17.9 x 10^2 CFU on orange peels.
Presence of *Bacillales* and *Actinobacteridae* represents and indicator of a good composting conditions (Partanen et al., 2010). *Bacillus* spp. is the dominant microorganism during the first stages of composting process (Awasthi et al., 2017; Biswal et al., 2009). Sundberg et al. (2013) demonstrated that lactic acid bacteria (LAB) and *clostridia* are responsible with high-odour in food waste composting sample. Liu et al. (2015) identified fungi like *Saccharomyces*, *Candida* and *Schizosaccharomyces* in different stages of the composting process and bacteria from the *Bacilli* class: *Bacillales* and *Lactobacillales* orders and from the *Actinobacteria* class: *Corynebacterinae* order using metaproteomics as identification technique. At the thermophilic stage of the composting process, bacteria belonging to the *Firmicutes* and *Proteobacteria phylae* were identified by Wang et al. (2017), while in the stabilisation stage Bernal et al. (2009) found that *Actinomycetes* is predominant.
Before composting begins solid waste should be analyzed. Therefore, the main purpose of this paper is to determine pH, electrical conductivity, moisture and N content of food waste (fruits and vegetables) and to identify and count colonies of microorganisms present on their surface. In this study it was also proposed to establish the relations between the microorganisms and the physico-chemical indicators studied.

2. MATERIALS AND METHODS

Samples collection:
Food waste samples (fruits and vegetable waste, peels and leaf) were collected from different households and were transported under appropriate conditions to the laboratory of Faculty of Food Engineering where they were analyzed.
Physico-chemical analysis:
The food waste samples were prepared according to the method presented by Nasreen and Qazi (2012) in order to determine pH and electrical conductivity (EC). IQ240 pH Meter and CyberScan CON 510 Conductometer were used to obtain the values for pH and EC. For determination of moisture (M) an oven *Nabertherm* was used. All samples were dried at 105 ^oC for 24 h until constant weight. Nitrogen (N) content from each sample was determined by using DK Series Kjeldahl Digestion Units - VELP Scientifica.
Microbiological analysis:
In this study spread plate techniques were used for microbiological assessment. Fruits and vegetables waste were placed in bags with sterile distilled water and homogenized using a BagMixer (InterScience). 1 ml of filtered and serially diluted (1/1000) homogenate was spread into medium plates under sterile conditions. The culture medium like Potato Dextrose Agar (PDA) for yeasts and Plate Count Agar (PCA) for total aerobic counts, were purchased from Sigma Aldrich. The incubation time was 48 h and after that the colonies were counted and the results were calculated considering the weight of the sample and dilution factor.

3. RESULTS AND DISCUSSION

In the first stage of this study were determined physico-chemical parameters in order to see the connection between the microorganisms and them. The obtained mean values of pH, EC, M and N content were illustrated in Fig. 1. pH values are between 4 and 7, the lowest pH was obtained for orange peels while the highest pH was determined for potato peels (Fig. 1a).



a) b)
Fig. 1. Mean values of: a) pH and EC; b) M and N for food waste samples

These values are quite similar with those obtained by Nasreen and Qazi (2012), excepting pH of apple waste. The lowest electrical conductivity was recorded for the cabbage leaves, while the highest value for conductivity was obtained for the potato waste followed by the banana peels (Fig. 1a). Results showed that all analyzed waste samples have high moisture, which has also observed in other studies (Asquer et al., 2013). Cabbage leaf has the highest moisture (91%), followed by carrot peels (90%) and banana peels (88%), while the lowest moisture was registered for orange peels (Fig. 1b). These values are also similar to those presented in other studies (Deressa et al., 2015; Nasreen and Qazi, 2012). The values obtained for the nitrogen content of the analyzed wastes are small, below 1% (Fig. 1b).

In the second stage of this study several colonies of microorganisms have been found on fruits and vegetables waste, thus:

- on apple peels and core: yellow, glossy, opaque, ridged colonies - G(-) cocci arranged in bunches (staphylococci); round, smooth edges, creamy, glossy, concave profile, opaque colonies - G(+) cocci placed in bunches (staphylococci) (Fig. 2a), round colony, jagged edges, translucent, glossy, crateriform, mucoid colonies - bacilli G (-); oval, creamy, glossy, opaque, smooth edges, developed in the depth of the environment, convex colonies - G (+) bacilli;
- on banana peels: colored punctiform, glossy, white, opaque, oval colonies - G (+) bacilli, simple; round, glossy, smooth, white, opaque, concave colonies - G (+) bacilli disposed simply or in diplo (Fig. 2b);
- on orange peels: round, white, glossy, convex, smooth colonies - G (+) staphylococci (Fig. 2c); round, straw-yellow, glossy, opaque, convex, smooth colonies - G (+) simple bacilli; irregular shape, creamy, glossy, concave, smooth colonies - simple G (+) bacilli etc.
- on kiwi peel: round, slightly irregular, creamy-brown, glossy, opaque, smooth yeast colony (Fig. 2d) or white-gray, round, glossy, convex, smooth yeast colony.
- on cabbage leaf: round, glossy, full margin, concave, crateriform, white, opaque colonies - sporulated G (-) bacilli in chains (Fig. 3).

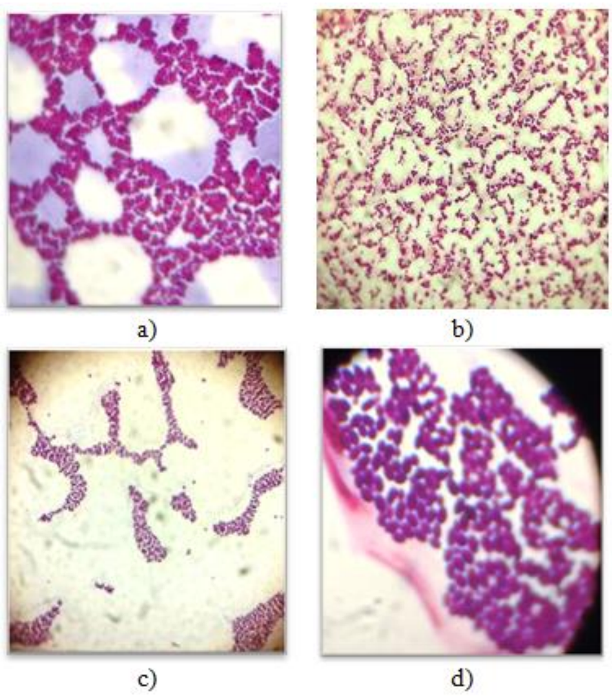


Fig. 2. Different colonies on fruit waste: a) staphylococci on apple peel and core; b) simple bacilli on banana peel; c) staphylococci on orange peels; d) yeast on kiwi peels

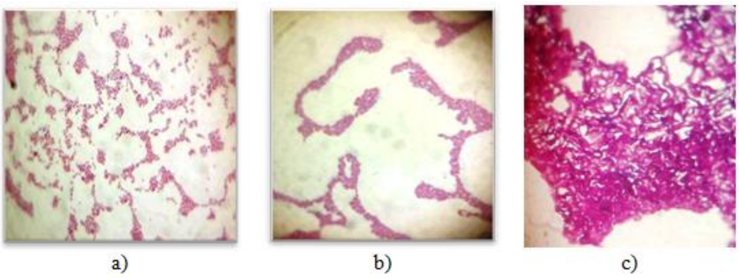


Fig. 3. Different colonies on vegetable waste: a) simple bacilli on potato peels, b) simple bacilli on carrot peel c) sporulated bacilli on cabbage leaf

- on potato peel: round, convex, creamy, glossy, opaque, smooth, uniform margins colonies - G (-) staphylococci; round, crateriform, gray-brown, glossy, opaque colonies - simple G (+) bacilli and others.
- on carrots: white, round, crateriform, glossy, opaque, rough colonies - G (+) bacilli, round, creamy, irregular, shiny, opaque, convex colonies - G (-) staphylococci.

After counting and calculating colonies, the results were expressed as log colony forming units (CFU) / g of food waste (Fig. 4).

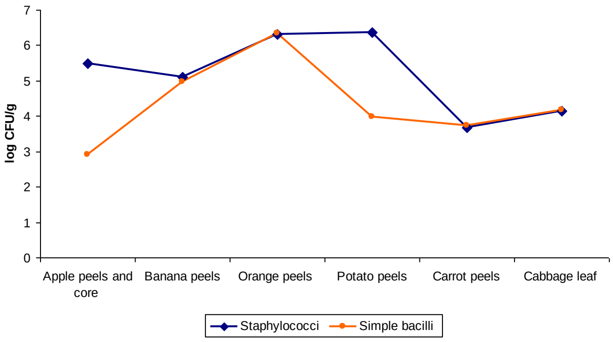


Fig. 4. Microorganisms found in food waste samples

A large number of staphylococci colonies were identified on potato waste and the fewest colonies on carrot waste. On orange peels were observed the largest number of simple bacilli, while on apple peels and core the fewest colonies compared to those found on the other types of waste analyzed. According to literature studies (Ahmed et al., 2007; Liu et al., 2015), bacilli are often encountered both before the start of the composting process and at different stages of this process. Hefnawy et al. (2013) observed that at the beginning of composting among the mesophilic bacteria found there were also species of *Staphylococcus* and *Bacillus*. Raja et al. (2012) used *Bacillus* spp. as culture for fruits and vegetable waste composting. Only on kiwi waste were found yeast (2.44E+06 CFU/g).

Poisson regression analysis was used to assess relationship between microorganisms (staphylococci and simple bacilli) and physico-chemical indicators like pH, M, EC and N content. Results showed that all four predictors (pH, M, EC and N) are statistically significant at the 0.05 level (p-value = 0.000) (Table 1).

Table 1. Deviance Table

Source	DF	Adj Dev	Adj Mean	p-Value
Poisson regression analysis: staphylococci versus pH; M; EC; N				
Regression	4	7536311	1884078	0.000
pH	1	347200	347200	0.000
M	1	403184	403184	0.000
EC	1	264552	264552	0.000
N	1	125076	125076	0.000
Error	1	2699	2699	
Total	5	7539010		

Source	DF	Adj Dev	Adj Mean	p-Value
Poisson regression analysis: simple bacilli versus pH; M; EC; N				
Regression	4	6934786	1733697	0.000
pH	1	136065	136065	0.000
M	1	55016	55016	0.000
EC	1	15197	15197	0.000
N	1	59277	59277	0.000
Error	1	30475	30475	
Total	5	6965261		

Deviance R^2 is 99.96% for staphylococci, and respectively 99.56% for simple bacilli which means that the model fits the data well. For the regression equations it was assumed that: staphylococci = exp(Y') (Eq. 1) and simple bacilli = exp (X') (Eq. 2).

$$Y' = 34.3931 + 3.7358pH - 0.6671M + 0.0038EC + 33.677N \quad (1)$$

$$X' = 7.225 - 7.8454pH + 0.8369M - 0.0022EC - 73.796N \quad (2)$$

4. CONCLUSION

In this paper were determined the microbiological and physico-chemical parameters of fruits and vegetables waste which will be subjected to composting. Results showed that pH values varies between 4 and 7, moisture is high and low values for nitrogen content were recorded. Regarding the microbiological activity it was identified colonies of simple bacilli and staphylococci. With the help of Poisson regression analysis it was established a relationship between microorganisms and physico-chemical indicators. In the future studies other type of microorganisms will be evaluated and different species will also be identified.

5. REFERENCES

[1]. Ahmed M., Idris A., Syed O.S.R. 2007. Physicochemical characterization of compost of the industrial tannery sludge. J. Eng. Sci. Technol. 2, 81-94.

[2]. Amir S., Hafidi M., Merlina G., Revel J.C. 2005. Structural characterization of fulvic acids during composting of sewage sludge. Process Biochem. 40, 1693-1700.

[3]. Asquer C., Pistis A., Scano E.A. 2013. Characterization of fruit and vegetable wastes as a single substrate for the anaerobic digestion. Environ. Eng. Manage. J. 12, S11, 89-92.

[4]. Awasthi M.K., Selvam A., Lai K.M., Wong J.W.C. 2017. Critical evaluation of post-consumption food waste composting employing thermophilic bacterial consortium. Bioresour. Technol. 245, 665-672.

[5]. Bernal M.P., Alburquerque J.A., Moral R. 2009. Composting of animal manures and chemical criteria for compost maturity. Bioresour. Technol. 100, 5444-5453.

[6]. Biswal B.K., Tiwari S.N., Mukherji S. 2009. Biodegradation of oil in oily sludges from steel mills. Bioresour. Technol. 100, 1700-1703.

[7]. Bohacz J. 2018. Microbial strategies and biochemical activity during lignocellulosic waste composting in relation to the occurring biothermal phases. J. Environ. Manage. 206, 1052-1062.

[8]. Deressa L., Libsu S., Chavan R. B., Manaye D., Dabassa A. 2015. Production of Biogas from Fruit and Vegetable Wastes Mixed with Different Wastes. Environ. Ecol. Res. 3(3), 65-71.

[9]. Gajalakshmi S., Abbasi S.A. 2008. Solid waste management by composting: state of the art. Crit. Rev. Environ. Sci. Technol. 38, 311- 400.

[10]. Hefnawy M., Gharieb M., Nagdi O.M. 2013, Microbial diversity during composting cycles of rice straw. Int. J. Adv. Biol. Biomed. Res. 3, 232-245.

[11]. Liu D., Li M., Xi B., Zhao Y., Wei Z., Song C., Zhu C. 2015. Metaproteomics reveals major microbial players and their biodegradation functions in a large-scale aerobic composting plant. Microb. Biotechnol. 8, 950–960.

[12]. McKinley S.P. 2008. Physical Chemical Processes and Environmental Impacts Associated with Home Composting. University of Southampton. Thesis for the degree of Doctor of Philosophy.1-222.

[13]. Nasreen Z., Qazi J.I. 2012. Lab Scale Composting of Fruits and Vegetable Waste at Elevated Temperature and Forced Aeration. Pakistan J. Zool. 44, 1285-1290.

[14]. Partanen P., Hultman J., Paulin L., Auvinen P., Romantschuk M. 2010. Bacterial diversity at different stages of the composting process. BMC Microb. 10, 94.
[15]. Raja M.M., Raja A., Hajee M.S., Sheik M.A. 2012. Screening of bacterial compost from spoiled vegetables and fruits and their physiochemical characterization. Int. Food Res. J. 19 (3), 1193-1198.
[16]. Rich N., Bharti A., Kumar S. 2018. Effect of bulking agents and cow dung as inoculant on vegetable waste compost quality. Bioresour. Technol. 252, 83-90.
[17]. Sánchez Ó.J., Ospina D.A., Montoya S. 2017. Compost supplementation with nutrients and microorganisms in composting process. Waste Manage. 69, 136–153.
[18]. Sundberg C., Yu D., Franke-Whittle I., Kauppi S., Smårs S., Insam H., Romantschuk M., Jönsson H. 2013. Effects of pH and microbial composition on odour in food waste composting. Waste Manage. 33, 204-211.
[19]. Wang C., Dong D., Strong P.J., Zhu W., Ma Z., Qin Y., Wu W. 2017. Microbial phylogeny determines transcriptional response of resistome to dynamic composting processes. Microbiome. 5, 103.
[20]. Zhang B., Lye L., Kazemi K., Lin W. 2013. Development of advanced composting technologies for municipal organic waste treatment in small communities in Newfoundland and Labrador, Faculty of Engineering and Applied Science, Harris Centre - MMSB Waste Management Applied Research Fund 2012-2013.