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Published version:

DOI:10.3303/CET1758043

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CHEMICAL ENGINEERING TRANSACTIONS
VOL. 58, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Remigio Berruto, Pietro Catania, Mariangela Vallone

ISBN 978-88-95608-52-5; **ISSN** 2283-9216
DOI: 10.3303/CET1758043

Effect of Organic Fertilization on Capsaicin Content in Trinidad Scorpion (*Capsicum Chinese*) Peppers: Preliminary Results

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Capsaicin is a pungent capsaicinoid responsible for the “hot and spicy” taste of chili peppers and pepper extracts. It is a valuable pharmacological agent with several therapeutic applications in controlling pain and inflammation. Emerging studies show that it displays patent anti-tumor activity in several human cancers.
A mesocosm experiment was set for testing the influence of the organic fertilization on capsaicin content in *Capsicum chinense* cv Trinidad Scorpion. Pelletized organic fertilizer from the composted solid fraction of pig slurry (OF) was compared to a mineral fertilizer (MF) and a mixture of organic pellets and mineral fertilizer (OMF). The study investigated the effects of a single application of 2 different doses of nitrogen (38 kg ha^{-1} and 76 kg ha^{-1}). Peppers were grown and fertilized according to the different treatments and after 500 days from the seeding fruits were collected and their capsaicin content was detected by means of the High Pressure Liquid Chromatography (HPLC) analysis.
The study shows that the capsaicin content is significantly affected by both the different fertilizers investigated and the nitrogen application rate adopted. The OMF treatment gave the best results in terms of fruit production and led to a percentage of capsaicin five time higher than that obtained with the MF treatment.

1. Introduction

In Italy, as in other European countries, pigs farming are becoming increasingly specialized, leading to a reduction in the number of farms. At the same time, specialization into pigs production has led to a concentration of animal production on large farms in restricted areas. In these areas, the slurry management mainly consists in land spreading after pond storage. This practice could cause serious environmental problems including an excessive input of potentially harmful trace metals (Lu et al., 2010), an increase in nutrient loss from soils through leaching, erosion and runoff due to not considering the nutrient requirements of crops (Gomez-Brandon et al., 2008) and the emission of ammonia and GHG (Salazar et al., 2005).
Consequently, there is a growing need to adopt slurry treatment technologies to optimize the management of the large amount of manure generated and to reduce potential risks of environmental pollution. Solid-liquid separation can be an effective slurry treatment method for producing nutrient-rich organic solids and potentially reducing the nutrient contents and organic matter in the liquid phase (Brito et al., 2008). The liquid fraction (LF) is generally used in land applications in areas near the farm, while the solid fraction (SF) is exported to outside farmland areas. Consequently, in many regions with intensive livestock, cultivated area sufficiently close to the farm facilities is not enough to satisfy manure disposal requirements, thus increasing costs for storage and transportation.
A promising approach to rise the value of SF from pig slurry is pelletizing. This densification process increases the bulk density of SF from an initial value < 500 kg m^{-3} to a final value of > 1000 kg m^{-3} (Pampuro et al., 2013), thus reducing costs for transportation, handling and storage (Kaliyan and Vance Morey, 2009). Furthermore, Romano et al. (2014) showed that pelletizing allows homogenizing and further concentrating the

Please cite this article as: Pampuro N., Tebaldo V., Fabbri D., Calza P., Faga M.G., Cavallo E., 2017, Effect of organic fertilization on capsaicin content in trinidad scorpion (capsicum chinese) peppers: preliminary results, Chemical Engineering Transactions, 58, 253-258
DOI: 10.3303/CET1758043

nutrients of SF, improving the uniformity of its fertilizing and amending actions. This opens the possibility of creating a new market for pig slurry-derived pelleted fertilizers.
In recent years the interest on "hot" chili peppers has increased because of two main reasons. First, there is an increasing commercial request of more and more hot chili pepper. Secondly because studies investigating the active ingredients and mechanisms of action of capsaicinoids, among which capsaicin constitutes 50%, pointed out interesting pharmacological activities. Among these, Yang et al. (2010) reports that a daily intake of capsaicin reduces blood pressure by increasing the amount of NO (nitric oxide), a potent vasodilator. For this reason chili is recommended in the preventative care of infarction and cardiovascular disease. Moreover, recent studies have shown that capsaicin is able to lead to self-destruction some cancer cells (Lee et al., 2012).
According to Menichini et al. (2009), the content of phytochemicals (i.e. capsacinoids) in plant material is influenced by numerous factors such as climatic conditions, ripening time, genotype and cultivation techniques.
To our knowledge, the effect of the organic fertilization on the capsaicin content in chili peppers have not been reported. In relation to the importance of capsaicin for pharmacological aspects, the aim of this work was to evaluate the influence of the fertilization - organic *vs* mineral - on capsaicin content in Trinidad Moruga Scorpion (TMS) chili.

2. Materials and Methods

2.1 Pelletized organic fertilizer preparation

Pellets included in the experiment were made of composted pig slurry solid fraction (SSFC). The SSFC was obtained by composting in windrow 6,000 kg of pig solid fraction derived from a screw press separator. After the set-up, the windrow was placed on a concrete floor and the process was monitored for 130 days. To reduce the moisture content of the pig solid fraction, making the organic material suitable for pelletizing, windrow was composted with a turned strategy (Pampuro et al., 2016). During the experimental period, the windrow was turned six times: at day 7,16, 28, 35, 50 and 71.
The SSFC was pelletized using the mechanical pelletizer CLM200E (La Meccanica Srl, Padua, Italy). It was powered by a 0.37 kW electric motor. The organic pellets were manufactured by forcing the composted material through 6 mm diameters holes in a matrix pattern (Pampuro et al., 2017).
The pellets were analysed for pH, dry matter content (DM), total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitric nitrogen (NO_3^--N), C/N ratio, OM, total phosphorous (expressed as P_2O_5) and total potassium (expressed as K_2O). Value of pH was determined in a water-soluble extract 1:10 (w/w) using Hanna HI 9026 portable pH meter fitted with a glass electrode combined with a thermal automatic compensation system. DM was calculated by drying at 105 °C for 12 h and organic matter content by loss on ignition at 430 °C for 24 h (Navarro et al., 1993). Samples for TOC analysis were prepared by drying the samples at 105°C for 24 h, followed by treatment with sulphuric acid to eliminate any inorganic C, with subsequent analysis on an elemental analyser (Carlo Erba Instruments). TN and NH_4^+-N were determined using the Kjeldahl standard method. NO_3^--N was determined by ion chromatography in a 1:20 (w/v) water extract (Garcia-Gomez et al., 2002). After $HNO_3/HClO_4$ digestion, P_2O_5 was determined colorimetrically and K_2O by flame photometry (Garcia-Gomez et al., 2002). Table 1 reports the main chemical characteristics of the pellet.

Table 1: Main properties of the pellet included in the experiment. Mean value and standard error of 3 replicates.

Parameter	SSFC	
	Average	S.E.
Dry Matter (%)	85.4	0.7
pH	8.1	0.1
TN (%)	3.3	0.1
NH_4^+-N (mg kg^{-1})	672.0	10.5
NO_3^--N (mg kg^{-1})	1460.0	13.8
TOC (%)	36.9	0.4
C/N	11.2	0.3
OM (%)	63.6	1.5
P_2O_5 (%)	4.0	0.1
K_2O (%)	1.0	0.1

2.2 Mesocosm experiment

According to Mouradi et al. (2016), the TMS seeds were soaked for 24 hours in an infusion of chamomile, useful to disinfect the seeds, soften the seed coat (the protective skin that covers the seed) and stimulate germination. Subsequently, the seeds were placed inside a rigid and transparent plastic box (“Germination box”) and laid on several sheets of kitchen paper ("scottex method"). Non-calcareous water was added in order to wet the layers of paper. In this way, the chili pepper seeds are able to absorb slowly and with continuity the moisture by capillarity. In order to obtain a fast germination, the germination box has been located in the proximity of a heat source (heater) able to maintain the environmental temperature between 26 °C and 28 °C. This technique has allowed to obtain a rapid (on average 4-6 days) and uniform germination.
The germinated seeds were then transplanted into pots filled with soil for sowing.
After three months, a mesocosm experiment was set for testing the effect of the organic fertilization on capsaicin content in a glasshouse with controlled environment (25 °C and 80 % humidity) using a randomized block design with four replicates. As reported in Table 2, four treatments were included in the experiment.

Table 2: Main properties of the pellet included in the experiment.

TREATMENT	DESCRIPTION	MACRO NUTRIENTS SUPPLIED (kg ha^{-1})		
		N	P	K
OMF	Organic + Mineral Fertilization	76.6	84.7	65.8
MF	Mineral Fertilization	38.3	38.3	54.2
OF	Organic Fertilization	38.3	46.4	11.6
OF_2	Organic Fertilization (Double dose)	76.6	92.8	23.2

Each mesocosm was uniformly packed with 3 L of soil at a bulk density of 1,350 kg m^{-3} (Wu et al. 2011). All pots were then moistened with deionised water in order to reach 60% water filled pore space (WFPS). The amount of water to be added to each mesocosm was calculated on the basis of soil 70 % of the water holding capacity and corresponded to 670 ml per pot. Thereafter, soil water content was adjusted by adding water with a drop irrigation system (4 L min^{-1} during 10 minutes) every two to five days, according to the crop requirement. Mesocosms were manually fertilized comparing two different doses of nitrogen (38.3 kg ha^{-1} and 76.6 kg ha^{-1}).
In order to avoid additional variables, all analysed chili peppers were harvested 40 days after maturation (chili pepper has acquired a deep red colour, Figure 1) and 90 hours after the last irrigation (water stress produces an increase in capsaicin content).

Figure 1: Harvested TMS chili peppers.

2.3 Extraction and analysis of capsaicin

The most commonly occurring capsaicinoids are capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) (Bennett et al., 1968). In this study, we limited the analysis of capsaicinoid to capsaicin, the most common component.
The content of capsaicin was detected accurately by HPLC analysis (High Pressure Liquid Chromatography).
The TMS chili peppers were comminuted and subjected to the following method for the extraction of capsaicinoids:

- chili pepper in contact with 100 mL of EtOH for three days;
- filtering (putting aside the liquid) and subsequent addition of 50 mL of EtOH; three days of contact;

- repetition of the second step.

The solutions corresponding to the three extraction steps were mixed and evaporated at room temperature. The samples were then filtered through Millipore 0.45 µm filters and, finally, the residue was subjected to analysis by HPLC (UV-Visible detector). Pure capsaicin was used as standard (Figure 2).

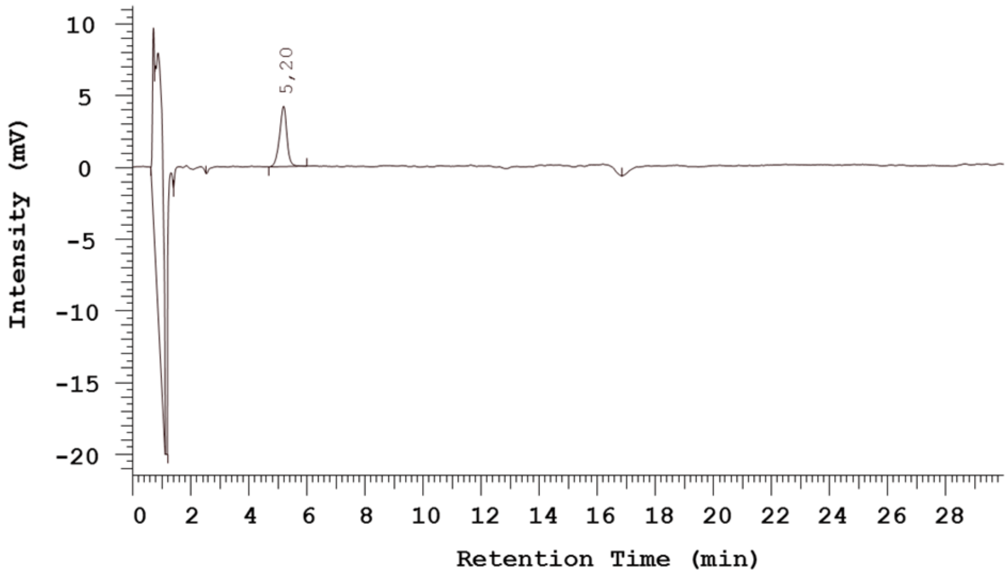

Figure 2: Chromatogram of the pure capsaicin.

The peak at the retention time of 5.20 min corresponds to capsaicin.

3. Results and Discussion

3.1 Agronomical aspects

The first difference between the various fertilization treatments, was noted in the period of flowering and subsequent fruit setting.

Generally the chili pepper plant regulates the production according to its nutritional availability, so its natural instinct will not ripen the fruit by dropping flowers, through the phenomenon of early drop.

In this study, during the development into fruit, early drop may have been determined by two main factors:

1. physiological early drop: the premature fall of chili pepper flowers could be due to the great amount of flowers produced by the TMS plants. Consequently, the plants are unable to lead such amount of flowers to fruits set;
2. nutritional deficiencies: the chili pepper flowers fall before fruit setting because they have a deficiency of nutrients. This problem could be solved by applying a fertilizer rich in potassium.

This phenomenon was more evident in OF and OF_2 which are the treatments with the lower concentration of potassium.

The second difference was the time required for fruit ripening and the size of the chili peppers. It is know that the yield of a plant is directly proportional to the volume of the pot in which it is grown; under the same conditions, chili peppers treated with MF were ripened four months earlier than those of the other treatments. Furthermore, MF treatment showed size and weight of the fruits significantly higher than the other treatments (Table 3).

Table 3: Average weight of the harvested TMS chili peppers. Mean value and standard error of 12 replicates.

TREATMENT	Fruit weight (g)	S.E.
OMF	2.194	0.129
MF	4.763	0.218
OF	2.632	0.065
OF_2	2.566	0.238

3.2 HPLC analysis

Figure 3 shows a chromatogram of TMS chili pepper (treatment OMF).

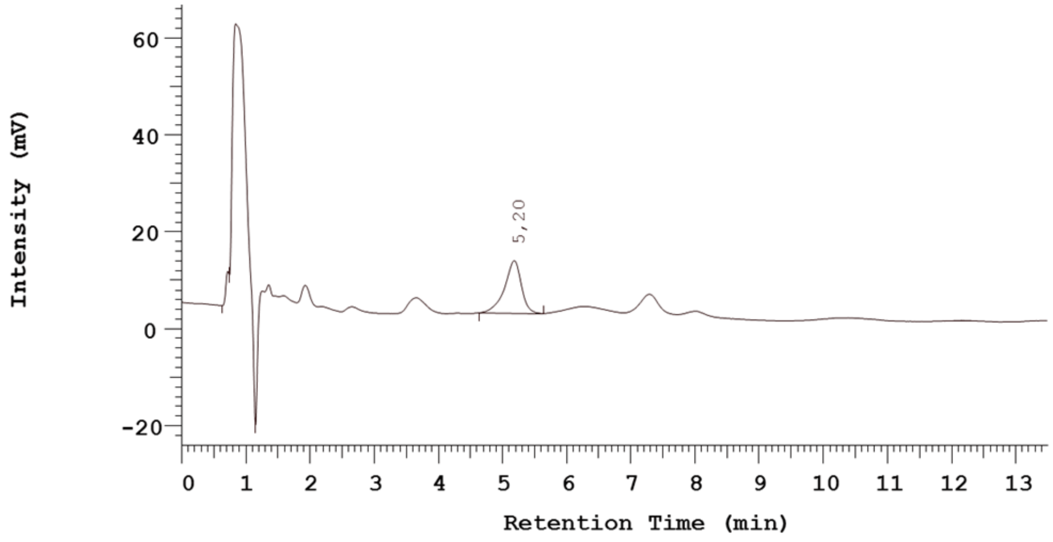

Figure 3: Chromatogram of TMS chili pepper - OMF treatment.

As presented in Figure 4, significant differences in capsaicin content (mg g^{-1}) were observed comparing the four treatments.

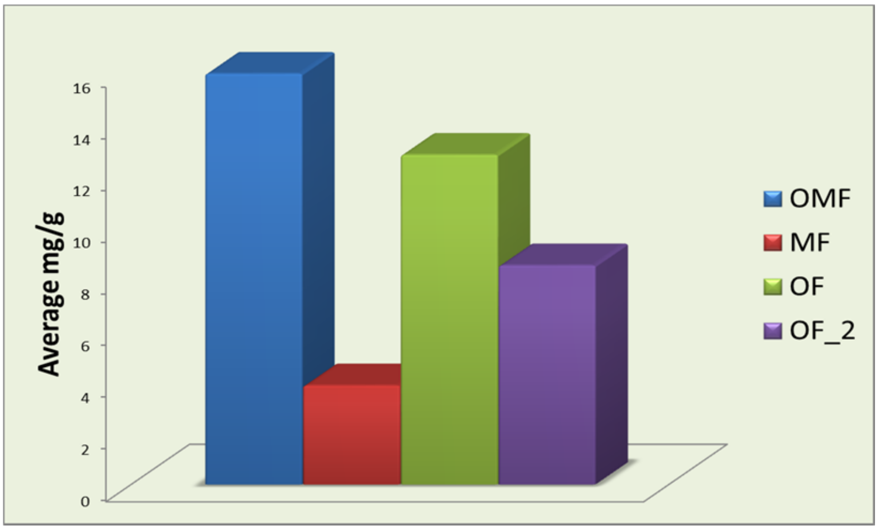

Figure 4: Average capsaicin content values (mg g^{-1}) in OMF, MF, OF and OF_2 treatments.

The results of the study show that:
- MF treatment leads to a rapid fruit setting, followed by a higher growth and by a rapid fruit ripening. This is probably due to the great amount of potassium in mineral fertilizer (Tassinari, 1968). At the same time, the early maturation led to a lower percentage of capsaicin within the chili peppers. Although OMF contains an amount of potassium higher than that present in MF, the rapid fruit ripening was not observed. This behavior could be related to the organic fraction present in OMF, responsible for the reduced speed of the nutrients release (Colea et al., 2016).
- The lower potassium content in OF treatment, has led to lower production of MTS fruits (the early drop phenomenon was significant). However, the grown and ripened peppers show a percentage of capsaicin four times higher than those produced in MF treatment.

- In OF_2 treatment, the concentration of capsaicin is lower than that obtained in the case of OF treatment, however is approximately double respect to that obtained in MF treatment.
- The use of a mixed fertilization (OMF treatment) produced a greater number of peppers but with smaller size if compared with the others treatments. However the MTS fruits presents the highest concentration of capsaicin.

4. Conclusions

The combined fertilization - mineral and organic - had significant effect on capsaicin content in MTS fruits giving the highest capsaicin concentration. These preliminary results are encouraging and they will be the basis for future research activities with the aim to individuate the best agronomical and environmental friendly practices able to concentrate the capsaicin in TMS chili.

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