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BioBoost

Accelerating biobased horticulture

Stabilisation methods and techniques

(WP3)

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ILVO
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BioBoost - Report Stabilisation Methods and Techniques

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Disclaimer
This report is a summary of the experiments carried out in the framework of the BioBoost project. Not all raw data are included in this report, but they are available for interested stakeholders upon request, if non-confidential.

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Content

General introduction

In an earlier phase of the BioBoost project, a literature study on stabilisation technologies relevant for horticultural biomass was performed in order to assess the state-of-the-art. This was done for the 15 most relevant crops selected from the BioBoost inventory[1]. For these crops also information on the nutritional composition of the food crops and their derived by-products and waste-fractions was collected. Based on interaction with farmers and industrial stakeholders, ILVO and VIVES selected a number of crops and by-products to evaluate specific stabilisation techniques as a first step towards improved utilisation of the horticultural biomass fractions. Besides the crops selected initially, the project partners detected additional opportunities to perform relevant, demand-driven stabilisation tests. An overview of all the stabilisation tests performed is given in table 1.

WP 3	Horticultural crop	Inventory	Literature stabilization technologies	Nutritional info*	Stabilization tests	Interested stakeholders*
1	Strawberry	✓	✓	✓	No data	-
2	Raspberry	✓	✓	✓	No data	-
3	Blackberry	✓	✓	✓	No data	-
4	Peas	✓	✓	✓	Cooling	Vegetable processing industry
5	Grean beans	✓	✓	✓	Cooling	Vegetable processing industry
6	Basil	✓	✓	✓	Drying	Frozen vegetable industry
7	Parsley	✓	✓	✓	Drying	Frozen vegetable industry
8	Bell pepper	✓	✓	✓	Fermentation	Farmer
9	Tomato	✓	✓	✓	Heating	Farmer, auction
10	Lettuce	✓	✓	✓	No data	-
11	Belgian Endive	✓	✓	✓	Heating, drying	Grower, fresh cut industry
12	Brussel Sprouts	✓	✓	✓	Heating	Grower, fresh and frozen processing industry
13	Cauliflower	✓	✓	✓	Fermentation	Fresh and frozen processing industry
14	Apple	✓	✓	✓	No data	-
15	Pear	✓	✓	✓	No data	-
16	Brewer's spent grain	X	X	X	Cooling	Brewing industry
17	Tomato crop residue	X	X	X	Fermentation	Growers

*Due to confidentiality restrictions the names of the individual stakeholders involved in the different stabilisation tests cannot be revealed.

Table 1: Overview of stabilisation tests performed in WP3

In this report we focus on the stabilisation technologies evaluated. Additional steps in process and product development, such as recipe development and the use of additional unit operations including preprocessing, value-added processing and conservation treatments will be described in a separate report that will be delivered towards the end of the BioBoost project.

Fresh fruits and vegetables are vitally important in the human diet as they are a primary source of proteins, carbohydrates, vitamins, dietary fibers, minerals, and minor but important bioactive nutrients such as e.g. polyphenols. One of the most limiting factors in marketing of fresh fruits and vegetables is their short shelf life. They are highly perishable due to the biochemical reactions

[1] This is a separate report of the BioBoost project, published on its website.

involved in metabolism, risk of infection with pathogenic microorganisms and environmental conditions of storage. If the harvested fruits and vegetables are not instantly processed and preserved using proper methods, the economic loss resulting from their spoilage can be substantial. In order to meet the increasing consumer demand for fresh-like, natural, and additive free and minimally-processed fruits and vegetables and to reduce economic loss, various processing and preservation technologies have been extensively investigated to extend the shelf life and to preserve the quality of fresh fruits and vegetables. Many physical preservation methods such as freezing, canning, and drying that rely on heating and cooling operations have been explored. Although these technologies ensure a high level of food safety, the heating and cooling of fruits and vegetables can result in significant quality losses. For instance, the colour, flavour, and texture of fruits and vegetables subjected to heating and/or cooling processes can be irreversibly altered (Li et al. 2017).

In the next chapters, a summary of the stabilisation test performed in the framework of the BioBoost project is described.

1. Thermal treatments

1.1 Introduction

Despite the recommendations of international health organisations and scientific research carried out around the world, consumers do not take in sufficient quantities of healthy fruit and vegetable products. In order to boost the consumption level of fruits and vegetables, the use of preservation and processing methods creates a unique opportunity for food manufacturers to provide additional products in which nutrient content is retained to a level similar or close to that found in fresh fruits and vegetables. For centuries, storage and preservation technologies have been utilised to transform these perishable fruits and vegetables into safe, delicious and stable products. Food preservation aims primarily to create a microbiologically safe product, but processors also strive to produce the highest-quality food. Depending on how processing is carried out, processing may result in a change in colour, texture, flavour and nutritional quality. Processing also provides numerous possibilities to turn the available biomass of fresh products that is not meeting the strict quality requirements for the fresh market into healthy processed products (Barett et al. 2012).

Thermal treatments are one group of often used processing/preservation methods that can be applied to fruit and vegetable biomass. Thermal processing is one of the most common current forms of food preservation because it efficiently reduces microbial population, destroys natural enzymes (PPO, PME, POD, LOX, …) and renders horticultural products more palatable. Most canned and bottled fruits and vegetables are produced under conditions of commercial sterility, and have a shelf life of 2 years or longer. Thermal processing essentially involves either heating unsterile foods in their final containers (canning), or heating foods prior to packaging and then packaging under sterile conditions (aseptic processing). When food is heated, chemical reactions are accelerated and new components related to colour and flavour can be produced and beneficial components can be lost. Accordingly, food composition changes and unique colour and flavour are created (Rawson et al. 2011; Barret et al. 2012).

1.2 Aim

Different thermal treatments were applied to selected (by-)products in order to verify the impact of the applied thermal processes on the most obvious relevant macroscopic quality parameters of the treated biomass. As indicated in Table 1: Overview of stabilisation tests performed in WP3
, biomass of savoy cabbage, tomato, Belgian Endive and Brussels Sprouts, were used in these tests.

1.3 Methods

a. Savoy cabbage

In some periods of the production season there might be surplus product or produce that doesn't meet the quality criteria for the fresh market (size, colour, ….). These biomass fractions might still be suitable for processing into a processed, convenience product like a puree for example.

Cutting – mixing: Firstly, whole heads of savoy cabbage were cut (each head in four pieces) and frozen overnight in a freezer at -18°C. After freezing overnight, one half of the cabbage which was meant to undergo a 70°C heat-treatment (now -5°C), was minced using a vacuum-cooking cutter. This was done in batches of approximately 10 kg, at a cutting speed of 6000 rpm. Each batch was minced for 3 minutes with or without additional heating (depending on the temperature after 2 minutes), until the puree reached a temperature of 0-5°C. This resulted in 36,7 kg of savoy cabbage

puree. This process was later repeated for the second half of the cabbage meant to undergo a 90°C heat-treatment. Samples of the raw puree were also taken for investigation of shelf-life.

Figure 1: Starting material (left), cutting process in vacuum-cooking cutter (middle) and pureed savoy cabbage (right)

Pressing with spiral-filter press (VaculIQ®): To obtain a puree with a suited consistency for later heat-treatment, the puree was separated into a pomace (solid) and a more liquid fraction. The product was pushed through a spiral filter combined with a vacuum two separate times, first using a 1000 µm filter and secondly using a 800 µm filter, before it reached a good consistency. The yield for the 70°C puree was ± 60,68% (22,27 kg 'juice'). After taking samples from the untreated product, 20,75 kg of puree was available for UHT-treatment.

The material for the 90°C-treatment also passed through the spiral-filter press twice, both times using a 800 µm filter. It had a yield of ± 57,85% (21,23 kg 'juice'). After taking samples, 19,75 kg was available for the UHT-treatment.

Heat treatment of the obtained puree with UHT system: The resulting material after using the spiral-filter press was subjected to a heat treatment using a UHT-installation. One batch of the filtered savoy cabbage puree was to be treated with a temperature of 70°C and a holding time of 2 min. Due to a build-up of pressure in the tubes and a slower flow of the product, holding time was eventually prolonged to 3 min. This is most probably the result of a gelling effect in the material during the heating process. The second batch of savoy cabbage puree was treated with a temperature of 90°C for a holding time of 1 min. There were no similar problems during this treatment, probably due to the shorter holding time.

For both experiments, the heat-treated puree was distributed in sterile bottles (500 ml) in a laminar flow. The bottles were immediately prepared for storage: most of the puree was stored in a cooling cell (4°C) until they were analysed.

Analyses

Macroscopic parameters

Colour

The colour of the samples were analysed using a Bench-Top Konica Minolta Colorimeter CR-5. Results were formulated as coordinates on the CIELAB-colour space (L* for black/white, a* for red/green, b* for blue/yellow). For each measurement, 3 separate petri dishes were carefully filled with the

cabbage puree, after which each petri dish was measured 3 times. The measurements were done immediately after preparation, after 1 day, 1 week, 2 weeks and 4 weeks of being stored at 4°C.

The mean value was used for further calculation of colour difference (ΔE), with the formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The colour difference was calculated to evaluate how the samples changed during storage, but also to compare the colour traits after being subjected to different heating conditions.

Moisture content and Bostwick value

Apart from colour, moisture content and Bostwick values were also followed during 4 weeks of storage at 4°C. Moisture content was measured with a Moisture Analyzer (Mettler Toledo), at 105°C. Bostwick value was determined in duplicate using a Bostwick meter (30 cm) and timing for 10 seconds.

Microbiology

To assess the microbiological safety and stability of the samples, routine microbiological tests were carried out on the fresh and stored samples. The following tests were performed:

- Total aerobic plate count (30°C)
- Total anaerobic plate count (37°C)
- Aerobic spore forming bacteria (30°C)
- Anaerobic spore forming bacteria (37°C)
- *Listeria spp*.
- *Listeria monocytogenes*
- Lactic acid bacteria (37°C)
- *Enterobacteriaceae* (37°C)
- Yeasts (25°C)
- Moulds (25°C)

For the analyses after 2 and 4 weeks of storage, only the first 4 were conducted and included in the report.

b. Tomato

For the tomatoes the goal of the stabilisation experiments was to fractionate the fresh tomatoes into a juice and a press cake fractions with maximal yield for the juice fraction. The thermal treatments applied were to find out which treatments were suitable to produce a safe tomato juice with a good physicochemical stability and shelf life. As a feedstock three different types of tomato were used: regular type (individual tomatoes), cluster type and beef type tomatoes. In addition a blend of these three was used as well.

Cutting – milling: Just before the start of the experiment the tomatoes stored at 4°C were cut and mixed using the Multicut 1500 system (Bruckner Liquid Food Tech, Vaculiq). During cutting and milling with this system, N_2 –gas was applied to avoid oxidation.

Pressing with spiral-filter press (VacuIIQ®): To obtain a juice with a consistency suitable for later heat-treatment, the cut and mixed tomatoes were separated into a pomace (solid fraction) and a liquid fraction. The product coming out of the Multicut was pushed through a spiral filter combined using a 600 µm filter. For every feedstock about 80 l of juice was collected in a vacuum vessel directly connected to the VacuIIQ.

Heat treatment of the tomato juice with UHT system: The resulting juice was subjected to a heat treatment using a UHT-installation available at Food Pilot. From the vacuum vessel it was pumped into a vessel under atmospheric pressure, which was coupled to the UHT System (APV SPP, SPX

Corporation, Gatwick, UK). The juice was subjected to a heat treatment at 90°C for 30 s after a pre-heating with degassing at 60°C at a flow rate of 100 l/h. After cooling below 10°C the juice was manually filled under hygienic conditions in a flow into PE-bottles of 0.5L. Samples were stored at 5-7°C and at room temperature (20°C)

Figure 2: Starting material in the Multicut before cutting & milling (left), juice extraction in the VacuIIQ (middle) and tomato press cake coming out of the VacuIIQ as solid fraction (right)

Analyses

Macroscopic parameters

Basic macroscopic parameters (pH, colour, Brix, density) of similar tomato juices were determined earlier and communicated to the client in a confidential report.

Microbiology

To assess the microbiological safety and stability of the samples, total aerobic plate count (30°C) as general routine microbiological test were carried out on the fresh and stored samples.

c. Belgian Endive

During the production of Belgian Endive, especially in soil-based production systems, the appearance of Belgian Endive chicons that do not meet the strict quality criteria put forward (e.g. Flandria label) is unavoidable. Chicons might be oversized, bent, have a deviating size, shape or a chicon head which is not properly closed, making them unsellable in the fresh market segment (see Fig. 3). However, despite their aesthetic defects, this products are of excellent quality, taste, safe etc. That's why we believe they deserve to be valorised into food products rather than bringing them back on the field, compost them or use them as feed.

Besides class II chicons, Belgian Endive growers also have a second by-product at their production sites, being the outer leaves of the chicons that are removed during cleaning and size classification.

Figure 3: Class II Belgian Endive chicons not suitable for the fresh market (left); Belgian Endive chicon with a head that is not properly closed (middle left); Belgian Endive by-products at production facility, besides the forced roots, individual Belgian Endive leaves make up most of the generated by-products (middle right); collected individual leaves (right)

As both the Class II chicons and the individual Belgian Endive leaves have a very high moisture content (around 95%), fast stabilisation and processing is of crucial importance in order to be able to valorise them into high added value applications. A first goal of the stabilisation experiments with Belgian Endive biomass therefore was to fractionate the wet biomass again into a liquid and solid fraction that are suitable to be evaluated as intermediate products in further process and product development in the other WPs of BioBoost. In second approach we evaluated the application of a heat treatment to the chicons and leaves directly.

I. First approach: fractionation into liquid and solid fraction

Cutting – milling: Just before the start of the experiment about 100 kg of Belgian Endive chicons stored at 4°C were cut and mixed using the Multicut 1500 system (Bruckner Liquid Food Tech, Vaculiq). During cutting and milling with this system, N_2–gas was applied to avoid oxidation.

Pressing with spiral-filterpress (VacuIIQ®): To obtain a juice with a consistency suitable for later heat-treatment, the cut and milled chicons were separated into a pomace (solid fraction) and a liquid fraction. The product coming out of the Mulitcut was pushed through a spiral filter combined using a 600 µm filter. About 90 l of juice was collected in a vacuum vessel directly connected to the VacuIIQ.

Heat treatment of the Belgian Endive juice with UHT system: The resulting juice was subjected to a heat treatment using a UHT-installation available at Food Pilot. From the vacuum vessel it was pumped into a vessel under atmospheric pressure, which was coupled to the UHT System (APV SPP, SPX Corporation, Gatwick, UK). The juice was subjected to a heat treatment at 95°C for 30 s after a pre-heating with degassing at 60°C at a flow rate of 100 l/h. After cooling below 10°C the juice was manually filled into sterile Bag-in-Box recipients of 3 litre. Samples were stored at 5-7°C and at room temperature (20°C).

Figure 4: Starting material in the Multicut before cutting & milling (left); juice extraction in the VacuIIQ (middle) - the nice white colour of the juice in the extraction cell illustrates the power of the system, i.e. oxidation (browning) is avoided thanks to low-oxygen milling and pressing strategy; Belgian Endive chicon's press cake coming out of the VacuIIQ as solid fraction (right)

II: Second approach: direct heat treatment applied to chicons and leaves

Cutting: both chicons and individual leaves were cut into pieces of about 1-1,5 cm, using a Cubes – stripes cutter (ARGON Type 410).

Heat treatment of the cut Belgian Endive chicons and leaves: After cutting about 0,5 kg of the chicons and leaves were immediately packed in vacuum bags. Different heat treatments were applied using a combisteamer (OES 20-10 GN, Convotherm) varying between a minimal treatment at 95°C for 5 min up to 100°C for 25 min. In each treatment 10 bags were included.

Analyses

I: First approach

Nutritional value

For both the raw starting material used, as well as the liquid and solid fractions obtained after pressing, a nutritional analysis was performed by an accredited commercial lab (LOVAP, Geel). Besides the macronutrients also mineral content was assessed by an accredited commercial lab (ECCA, Merelbeke).

Food safety

In addition, food safety parameters were assessed for both the raw starting material used, as well as the liquid and solid fractions obtained after pressing. Pesticides, mycotoxins and heavy metals were analysed by Primoris (Zwijnaarde).

II: Second approach

The heat treated products were assessed by three different persons evaluating taste, texture and visual appearance.

Microbiology

For both approaches the heat treated Belgian Endive products were assessed for microbiological safety and stability using the standard microbiological assays as described for the Savoy Cabbages.

d. Brussels sprouts

As is the case for most of the fruits and vegetables, also for Brussels Sprouts, there are strict quality requirements for the products allowed to be sold on the fresh market (size, colour, shape etc, e.g. described in Flandria label; Figure 5). As a consequence part of the harvest is not suitable, unsellable to the fresh market.

Figure 5: Brussels sprouts with deviating size, colour shape, not reaching the quality criteria for the fresh market

The idea of the stabilisation test performed here is to turn these rejected Brussels sprouts into a safe, stable, tasteful and easy to use food product by applying heat as a stabilisation treatment. Fig. nn describes the approach that was followed in the initial stabilisation test. The raw Brussels sprouts were blanched (5 min treatment) or cooked (15 min treatment) using two different ways of heating, i.e. either being submerged in boiling water or heat-treated using steam. After the heat treatment, the samples are mixed during 3 or 6 min to obtain a puree, which was used for further analyses. In the scheme below, the use of steam as heat treatment is illustrated (not the red star in the steam above the cooking kettle.

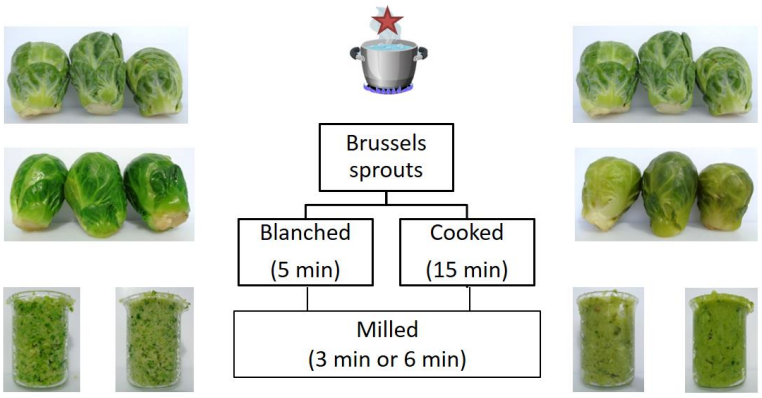

Figure 6: Approach followed to stabilize and process unsellable Brussels sprouts by heat treatment into a stable food product, i.e. Brussels Sprouts puree

Analyses

The first quality and stability parameters that were measured where the chlorophyll content as an indicator for the green colour of the treated samples and the residual enzymatic activity of the quality related enzymes peroxidase (POD) and polyphenol oxidase (PPO).

Total chlorophyll, chlorophyll a and chlorophyll b were determined according to the procedure of Armesto et al. (2017) with some modifications.

Both PPO and POD extraction and assay were performed according to the method reported by Yi et al. (2017).

1.4 Results

a. Savoy cabbage

Macroscopic parameters

Colour: The cabbage puree processed at both 70°C and 90°C always had a higher L*-value compared to the untreated, raw material (L*=45,64 and L*=45,11 vs. L*=39,13, respectively), initially after production and throughout 4 weeks of storage, meaning that the thermally treated puree had a darker colour. Immediately after processing the raw puree had a higher a* value then the purees thermally treated at 70°C and 90°C (a*= -13,93 vs a*=-18,62 and a*=-17,54, respectively), meaning that the untreated puree had a slightly brighter green colour then the thermally treated purees. However, this difference disappeared after one week of storage. In general, both for the raw puree as well as for the thermally treated purees, the a*-value increased during the storage period, e.g. from a*=-18,62 at the start up to a*=-2,88 after 4 weeks of storage for the puree treated at 70°C. Similar ΔE's were observed between the raw puree and the thermally treated purees, both at the start and during storage. The biggest ΔE's were found between the thermally treated samples measured immediately after processing and after one week of cold storage (ΔE's = 16,75 and 18,54 for purees treated at 90°C and 70°C, respectively.

Moisture content: The thermal treatment did not affect the moisture content in the purees. As for the raw untreated puree, the moisture content remained stable around 90,00 %.

Bostwick: Thermal processing resulted in an increase in the Bostwick value from 1 measured from the raw material up to 2,75 and 3,75 for the purees treated at 70°C and 90°C respectively.

Microbiology

As expected, the thermal treatment of 90°C resulted in the highest reduction of the microbial load. Looking at the aerobic plate count data, a 4 log reduction was achieved with the treatment at 90°C ($9x10^2$ cfu's) , while that at 70°C only resulted in a 3 log reduction ($6x10^3$ cfu's) compared to the microbial load of the raw material ($1x10^6$ cfu's). However, both thermally treated purees were microbiologically safe and stable throughout the 4 week storage period.

b. Tomato

Process parameters and juice yield

After optimisation of some process parameters (spiral used, 4 channels vs. 5 channels) high juice yields were obtained using the spiral-filter press:

Tomato type	**Regular type**	**Cluster type**	**Beef type**	**Blend of 3 types**
Juice Yield (%, w/w)	93	90	95	93

A degassing step during UHT treatment was crucial to increase the density of the juice, from ± 0.6 g/ml before, to ± 1 g/ml after degassing. Fresh tomatoes and its derived juice contain a lot of dissolved oxygen, the degassing step removes this and hence leads to an improved physicochemical stability of the juice. There's a slight colour difference between the juice derived from the regular type and cluster type tomatoes. The most obvious difference however is observable between the juice derived from the beef type tomatoes and the three other juices. The later has a paler red top phase, probably because less or smaller solid particles are present in this juice. Blending the three tomato types in juice production results in a juice looking similar as the juice produced from the cluster and regular type of tomatoes (Fig. 7)

Figure 7: Heat stabilized juices after one month storage at 5-7 °C, derived from 4 different tomato feedstocks

Microbiology

From the total aerobic plate count (30°C) carried out on the samples stored for 4 months at 5-7°C degrees, it was clear that all four juice types were microbiologically safe and stable following the heat treatment applied (90°C for 30 s, pasteurization conditions). The amount of cfu's counted in the total aerobic plate count varied between $5.3x10^1$ cfu's/ml and $1,9x10^2$ cfu's/ml.

c. Belgian Endive

I: First approach: fractionation into liquid and solid fraction

Juice yield

First of all, the fractionation process was very efficient, resulting in a juice yield of about 90%. The non-pasteurised juice oxidized very quickly when coming into contact with oxygen. In line connection to UHT for heat treatment is therefore crucial to preserve its initial colour and quality.

Nutritional value

In Table 2, the nutritional value of the Belgian Endive chicons as starting material and the derived fractions juice and press cake is depicted.

Parameter	Unit	Chicons	Juice	Press cake
vocht	g/100g	95.4	96.4	90.6
as	g/100g	<0.5	<0.5	0.70
eiwit (N% x 6,25)	g/100g	0.6	0.7	1.1
vet na zure hydrolyse (Weibull)	g/100g	<0.2	<0.2	<0.2
red. suikers na inversie (als glucose)	g/100g	2.9	2.9	3.3
voedingsvezels (Asp)	g/100g	<1.0	<1.0	4.5
totaal koolhydraten (berekend)	g/100g	4.0	2.9	3.3
energetische waarde	kcal/100g	18	14	27
energetische waarde	kJ/100g	78	61	111

Table 2: Summary of the nutritional analysis of chicons, juice and press-cake obtained after low-oxygen fractionation

From the results it's obvious, as expected, that you have a concentration effect of the dietary fibres ('voedingsvezels') in the press cake (4,5 g DF/100 g fresh material). There's little protein, no fat and about 3g/100 g of sugars in the products.

Regarding the mineral analysis, K is most important, and enriched in the press cake, as is the case for all other minerals (table 3).

		Chicons	Juice	Press cake
Parameter	**Eenheid**	**Resultaat**	**Resultaat**	**Resultaat**
Calcium (Ca)	g/100g	0.0199	0.0130	0.0358
Magnesium (Mg)	g/100g	0.00723	0.00547	0.0117
Kalium (K)	g/100g	0.186	0.173	0.251
Ijzer (Fe)	mg/kg	<3.0	<3.0	4.3
Totaal fosfor	mg/kg	170	180	260

Table 3: Summary of the results from the mineral analysis of chicons, juice and press-cake obtained after low-oxygen fractionation

Food safety

From the analysis of pesticides, mycotoxins and heavy metals it was obvious that in the juice produced none of these compounds was detected above the legal limits according to EU legislation. In the press cake however, traces of fluopyram (19 µg/kg) and cadmium (17 µg/kg) were detected. For cadmium the detected value is close to the tolerable weekly intake (TWI) for cadmium of 2.5 µg/kg body weight (EFSA 2009) and hence this deserves further attention in the development of valorisation strategies.

Microbiology

As is evident from the results of the microbiological analysis summarised in table 4 on the next page, it is clear that the thermal treatment applied resulted in a microbiological safe product. The aerobic germs on the feedstock where reduced with 5 logs. The lactic acid bacteria detected in the fresh, unpasteurised juice were also killed, as well as the yeasts (gist) and moulds ('schimmels') which are not detectable anymore in the pasteurised juice.

Parameter	Fresh leaves	Unpasteurized juice	Pasteurized Juice
Totaal aantal aërobe psychro-trofe kiemen bij 21°C	$2{,}8.10^5$ kve/g	$3{,}5.10^6$ kve/g	$4{,}6.10^1$ kve/g (geschat aantal)
Telling van melk-zuurbacteriën bij 21°C	< 10,0 (+14 schimmels en 13 gisten)kve/g	$5{,}7.10^3$ (+5 schim-mels en 2 gis-ten)kve/g	< 10,0 kve/g
Telling van gisten bij 25°C	$9{,}6.10^2$ kve/g	$3{,}4.10^3$ kve/g	< 10,0 kve/g
Telling van schimmels bij 25°C	$2{,}6.10^2$ kve/g	$2{,}0.10^2$ kve/g	< 10,0 kve/g

Table 4: Summary of the microbiological analysis of fresh leaves that were pressed into juice

II: Second approach: direct heat treatment applied to chicons and leaves

Visual and organoleptic evaluation

After the application of different heat regimes on both cut crops and cut individual leaves, a sensorial and visual evaluation was made. The scheme below (Fig. 8) gives an overview of the Belgian Endive products obtained after the thermal treatment. From this test it became evident that the lowest heat treatment resulted in darker, more brownish colour of the product. A slightly more intense heat treatment resulted in a brighter colour, while retaining a good texture of the Belgian Endive pieces. Based on these test, these conditions were determined as the optimal time-temperature combination to use sous-vide cooking as a thermal treatment to stabilize Belgian Endive chicons and leaves.

Microbiology

The chicon samples treated with the conditions considered as optimal were used to evaluate the microbiological stability during storage at 4°C, up to 3 months after processing. From the microbiological analyses of these samples it became clear that there was a slow but gradual increase of the aerobic and anaerobic psychotropic germs during storage (up to $9{,}7x10^4$ cfu/g). However at the end of the storage period these values were still below the legally defined target values. In addition also the visual and sensorial evaluation of the stored product indicated that it is still of good quality. In conclusion, the applied heat treatment resulted in a product of good organoleptic quality that can be stored up to at least 2 months (taking into account a safety margin of month).

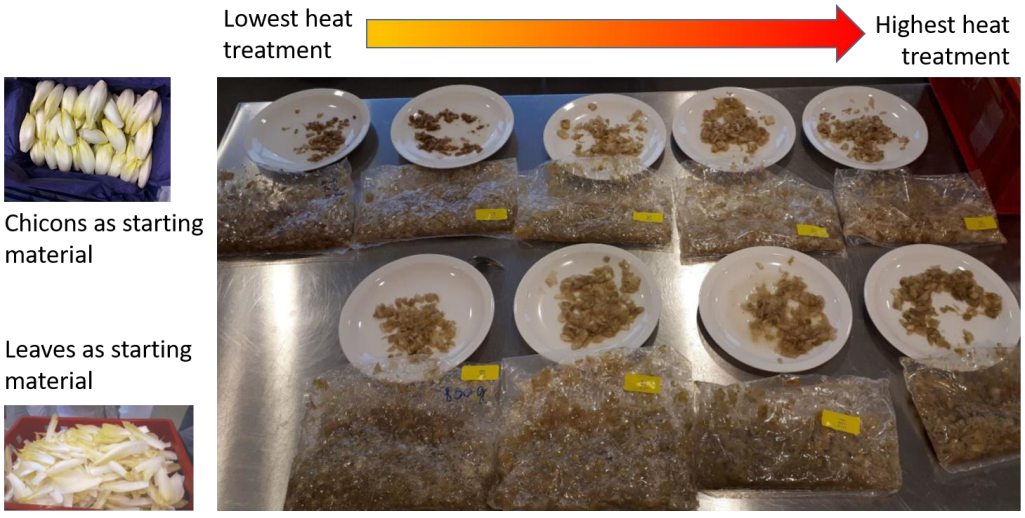

Figure 8: Result of the sous-vide cooking treatments on the colour and texture of cut Belgian Endive chicons and leaves

d. Brussels sprouts

Chlorophyll content

As explained above, total chlorophyll content (a+b) were measured in order to evaluate the impact of the heat treatments applied on the colour of the Brussels sprouts. As is evident from figure 9, both blanching and cooking had a significant impact when steam was used, which was not the case when boiling water was used to either blanch (5 min) or cook the Brussels sprouts (15 min). The impact of short (3 min) or longer mixing (6 min) of the heat-treated samples on chlorophyll measurements needs further investigation as no clear conclusions can be drawn based on the currently available data. In first instance the methodology of the analysis will be further validated as this was a newly established protocol.

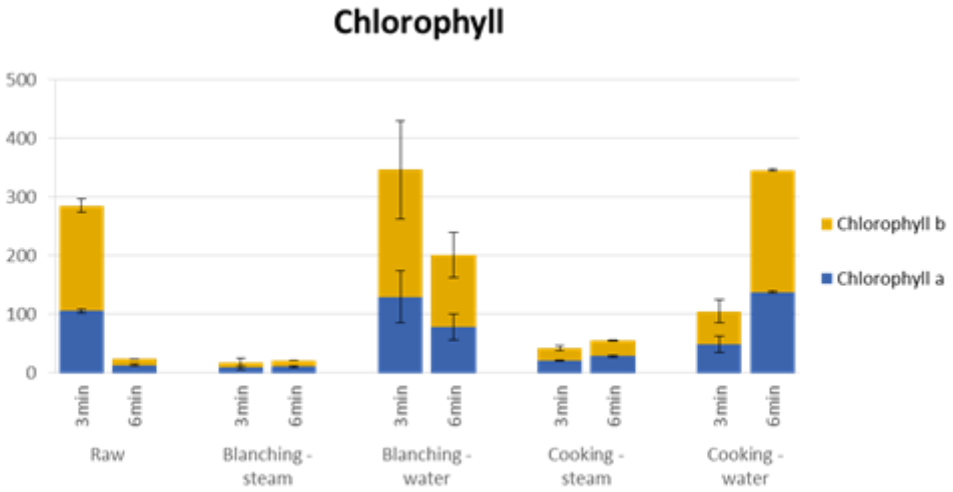

Figure 9: Chlorophyll content of mixed Brussels sprouts samples subjected to different heat treatments

Residual PPO & POD activity after thermal treatment

The residual activity of two relevant quality degrading enzymes were assessed as a parameter to evaluate the stability of the thermally treated Brussels Sprout samples. From figure 10 on the next page we can conclude both cooking treatments (15 min at 100°C), using either boiling water or steam totally inactivated both enzymes. Regarding the blanching treatment (5 min at 100°C), only the process using boiling water resulted in complete inactivation for both enzymes. The blanching method using steam resulted in the detection of residual enzymatic activity between 20-50% for PPO and between 40 and 70 % for POD. Again, the impact of shorter or longer mixing after the heat treatment on the determination residual enzymatic activity needs to be further investigated.

Nevertheless, based on the data collected in these experiments it can be concluded that the thermal treatments applied using boiling water resulted in the best colour and stability of the Brussels Sprouts.

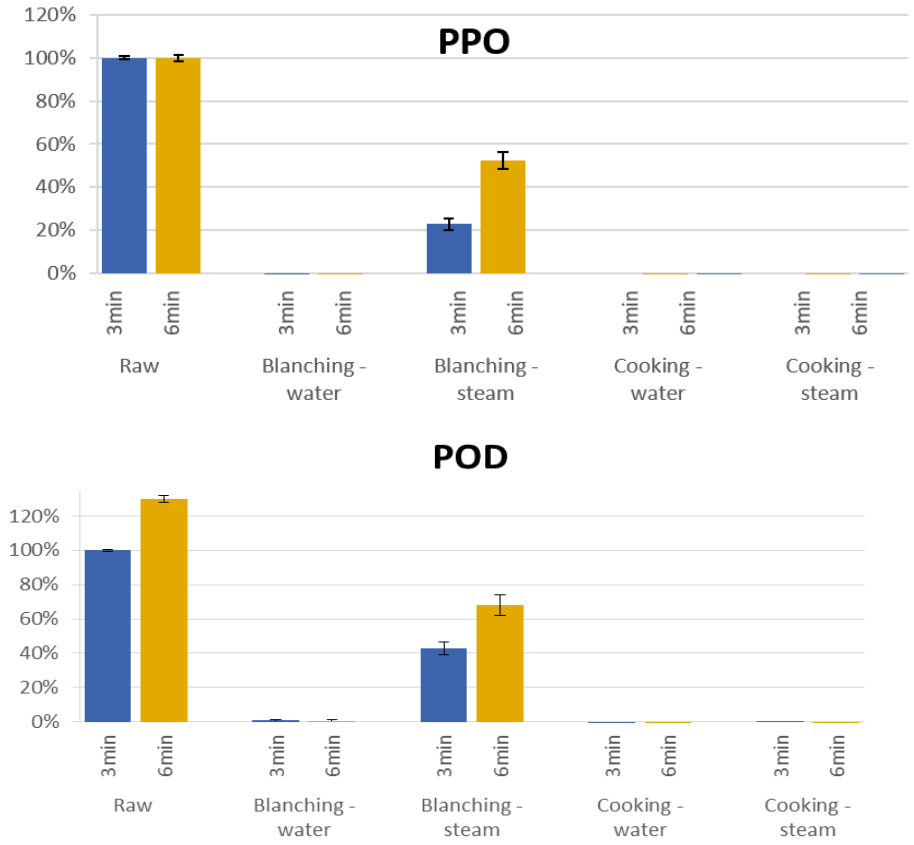

Figure 10: Relative PPO (upper panel) and POD (lower panel) activity of raw untreated, blanched and cooked Brussels sprout samples after mixing for 3 or 6 min

1.5 Discussion and conclusions

Thermal treatments are an important group of often used processing/preservation methods that can be applied to fruit and vegetable biomass. It efficiently reduces the microbial population present on the fresh produce, destroys quality affecting enzymes (PPO, PME, POD, LOX, …) and renders horticultural products more palatable.

In the work described in this first chapter of this report, different processing strategies including a thermal treatment were followed to process available by-products from different vegetables into stable, safe derived fractions that either can be used as a potential end-product or food ingredient.

Each raw food material such as vegetables and fruits and the by-products and waste fractions derived thereof, have their own, specific characteristics (pH, moisture content, aW, microbial load…). As a consequence, there is no 'one size fits all' processing strategy.

From the preliminary results included in this report it is evident that:

(1) there are significant volumes of vegetable by-products that can be processed into safe, stable food ingredients or end products.

(2) thermal treatments, applied in different ways, chosen in function of the product to treat, are effective as stabilization/conservation treatment for the processing of the by-products studied

(3) the knowledge and expertise to study the impact of thermal processing is available, and this can be used to further optimize the processing conditions.

2. Cooling techniques

2.1 Introduction

Cooling at refrigeration temperatures is a simple method to preserve fresh produce like fruits and vegetables. Traditionally, it is used as a treatment for moist products to remove field heat and thus extend shelf life. Consequently it is also relevant to be applied as a stabilisation technology to prolong the shelf-life of the crop's by-product and waste fractions that have the potential to be valorised in an application with higher added value as those that currently exist, being mainly (composting, bio-energy, feed). Different cooling technologies exist. In conventional cooling methods, such as air blast cooling, slow chilling and water immersion, cooling of a similar product can take up to five times longer compared to more innovative cooling technologies such as vacuum cooling (Li et al. 2017). In the test performed during BioBoost, conventional cooling was used.

In addition, freezing serves as a method of preservation because water activity can be lowered to a level which prevents microbial activity and reduces the rates of chemical reactions. There are three basic freezing methods used commercially: freezing in air, freezing by indirect contact with the refrigerant, and freezing by direct immersion in a refrigerating medium. Prior to freezing, most vegetables are exposed to a short blanching treatment with either steam or hot water to inactivate enzymes. While the thermal exposure in frozen vegetables and fruits is relatively low, the freezing and thawing process itself results in significant tissue structure damage, depending on the rate and temperature at which each is applied. This degradation of plant tissue may allow loss of cellular integrity and interaction of enzymes and nutrient substrates, resulting in nutrient loss in addition to deterioration of texture, colour and flavour.

2.2. Aim

Cooling tests were performed on waste streams that were planned to be used in product development. These waste streams were microbiologically analysed to establish the storage period at temperatures between 2°C and 5°C. At this moment, no by-products were selected for freezing test.

2.3 Methods

a. Green beans

The waste stream of the green beans was taken in the process where the stems and pieces with diverge colour are sorted out. It contains stems, small beans, some foreign objects such as corn, the head of a zucchini, but also beans with deterioration, brown spots, pale colour or dark green spots are present. The beans are already washed twice with recycled, non-drinkable water. One part of the sample was stored at 2°C, a second portion was stored at 5°C. After 1 day and 4 days of storage the samples were microbiologically analysed in double and sensorial evaluated.

b. Brewers' spent grain

Brewers spent grain was collected in the brewery immediately after filtration. The sample was divided in portions, stored at 2°C and microbiologically analysed up to 6 days after collection.

2.4 Results

a. Green beans

The green beans stored for 4 days at 2°C still meet the guidelines for yeasts of fresh unprocessed vegetables (Uytendaele et al., Microbiological guidelines). When stored at 5°C yeasts are growing and their number exceed the tolerance value (Fig. 11). At day 4 the sensorial properties (texture and smell) of the sample stored at 5°C were not acceptable anymore.

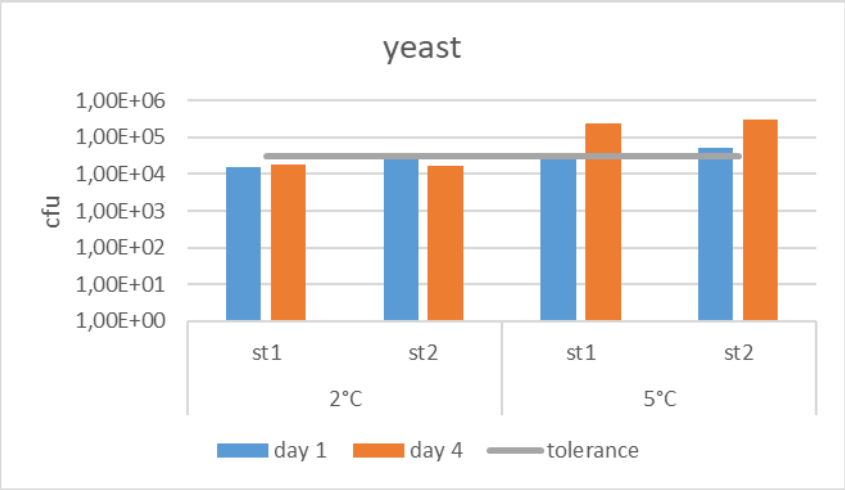

Figure 11: Yeast in green beans samples

b. Brewers' spent grain

Results of total aerobic psychotropic count and total anaerobic psychotropic count stayed below detection limit of 10 cfu/g. 40 cfu/g of yeast was found after 5 days of storage, and 10 cfu/g of mould was found after 2, 5 and 6 days of storage at 2°C. There was no visible formation of mould in the sample after 6 days.

2.5 Discussion and conclusions

a. Green beans

Microbiologically only the amount of yeasts are relevant as indicator for the quality as the aerobic count might vary depending on several conditions such as weather, harvest conditions, storage, therefor there is no tolerance indication on the graph of the total aerobic count. The guideline for moulds is that they should not be visible in the sample. As there were beans that were already deteriorating it was expected that the shelf life of this waste stream was going to be very short if the sample was not sorted out before storage in the fridge. In conclusion it is necessary to process the waste stream immediately after collection in order to have a clean product to work with. A storage temperature of 2°C is preferable.

b. Brewers' spent grain

Spent grain has a high moisture and fermentable sugar content, therefor it is said to be very unstable and liable to deteriorate rapidly due to microbial activity. The spent grain that was collected in brewery 'Halve Maan' can be stored for at least 6 days at 2°C .

3. Fermentation

3.1 Introduction

Fermentations processes are some of the oldest technologies in food, having been developed with the aim of increasing the storage stability of foods and improving the organoleptic and textural properties of raw materials. Fermented foods remain very popular even today as a substantial percentage of daily consumed foods are fermented. Some examples include dairy products, such as yogurt, cheese, buttermilk; alcoholic drinks, such as wine, beer and cider; fermented vegetables, such as sauerkraut and pickles; and fermented meats, such as sausages and salami.

Regardless of the raw materials used, vegetable fermentations can generally be described as a three-step manufacturing process, starting with a pretreatment, followed by the set-up of the fermentation conditions, and finalised by the actual fermentation (Li, 2004).

I. PRETREATMENT
Before fermentation, the vegetables undergo a pretreatment, which consists of their sorting and washing. Depending on the raw materials, other pretreatment operations, such as cutting (cauliflower, paprika, apples and tomatoes), slicing (cucumbers), peeling (carrots) are performed.

II. SET-UP OF THE FERMENTATION CONDITIONS
After the pretreatment, a suitable environment to select for the desired fermentation microbiota is set up. At the start of vegetable fermentations, Enterobacteriaceae, Pseudomonadaceae, yeasts, and moulds are the major constituents of the fermentation microbiota, whereas the number of LAB is minor. To favour the outgrowth of the outnumbered LAB and to inhibit the growth of spoilage and pathogenic microorganisms, salt (most often NaCl) is added to the raw vegetables (Li, 2004). This either occurs through the addition of dry salt (dry salting) or of a salt solution (brine) as mentioned in the literature study. Besides the salt concentration, other factors, such as the fermentation temperature, pH, and anaerobiosis play an essential role for the success rate of vegetable fermentations. Appropriate fermentation temperatures and anaerobic conditions will help to select for LAB and to inhibit the growth of undesired microbial communities (Li,2004). During fermentation, acidification, as a result of the growth of LAB, also enhances the selection for LAB and the inhibition of undesired microorganisms. Finally, to facilitate the domination of LAB in vegetable fermentations, the use of starter cultures is possible (Li, 2004).

III. ACTUAL FERMENTATION
Vegetable fermentations typically occur in different phases, each phase being characterized by its own prevailing microbiota. When the appropriate fermentation conditions are established, the undesired communities of Gram-negative bacteria, yeasts, and moulds are not able to persist and, as a result, are outcompeted by the LAB that acidifies the environment. The actual fermentation, dominated by LAB, is most often divided into two phases, of which the first phase is usually dominated by heterofermentative LAB species, such as *L. mesenteroides*, whereas the final phase is driven by more acid-tolerant homofermentative LAB species, such as *Lb. plantarum*. Depending on the fermentation, many other LAB species and yeasts can be involved in the different fermentation phases.

3.2 Aim

This study describes the spontaneous vs. induced fermentation of different vegetables and fruits (cauliflower, cucumbers, paprika, tomatoes and apples). More accurately the aim of this study was to assess the microbial ecology during spontaneous and culturing fermentation and achieve a stable end product ready to serve with the benefits of the fermentation properties. The last part of this study includes the fermentation of spent grain from brewery, having as an end product cookie with 50% of fermented spent grain. In this case, the aim of the fermentation is the storage of the spent grain and its use in bakery products as a fibre source.

3.3. Materials and methods

a. General

Material:
In order to carry on fermentation process the following material will be needed:

- Glass jar
- Airlock
- Labels
- Marker
- Spoons
- Ethanol for disinfection
- Starter cultures

Material disinfection:
Preheat the oven at 160ºC. Place the jars in the oven with the opening upwards for 15 minutes. Use the jars after the temperature is cooled down. The lids are sterilized by simply immersing them into the boiling water. The airlocks use disinfected by using alcohol and washed out with tap water.

Starter cultures used in the tests:

- Rye sourdough
- Kefir bioferment
- Cutting edge vegetable starter culture
- Oral probiotic starter culture
- Cheese whey
- Backslopping

b. Cauliflower

I: Optimal salt concentration spontaneous fermentation

Ingredients:

- 500 gr. of cauliflower
- Salt (the amount differs depending on the salt concentration of each recipe)
- 1L boiled water
- 1 cabbage

Procedure:

- Prepare the brine at 3 different concentrations of salt (4%, 10%, 16%) mixing 1L of boiled water with 40, 100 and 160 grams of salt respectively.
- Wash and cut the cauliflower in approximately the same sized pieces
- Place the cauliflower in the fermenting glass jar
- Add the brine and make sure the cauliflowers are fully submerged in the brine

- Place a plastic bag filled with water (the inner part of the plastic bag is in contact with the brine)
- Close the jar with the lid
- Place the airlock on the top of the lid
- Place the jars in a dark cupboard until the fermentation process is done is at room temperature.

II: Spontaneous vs. induced fermentation

Ingredients:

- 500 gr. of cauliflower per jar
- 40 grams of salt
- 1L boiled water
- Starter cultures

Procedure:

- Prepare the brine at 4% NaCl mixing 40 grams of salt in 1L boiled water. Mix it until the salt dissolves and add the starter culture. Mix again and let the brine rest for a couple of minutes before pouring in the jar.
- Wash and cut the cauliflower in approximately the same sized pieces
- Place the cauliflower in the fermenting glass jar
- Add the brine and make sure the cauliflowers are fully submerged in the brine
- Shape the cabbage leaves and place them on the top in order to keep the cauliflower under the brine
- Close the jar with the lid
- Place the airlock on the top of the lid
- Place the jars in a dark cupboard until the fermentation process is done is at room temperature.

c. Tomato

I: Cherry tomatoes with water bag on top

Ingredients:

- 1kg of cherry tomatoes
- 30 grams of sea salt

Procedure:

- Wash and cut the tomatoes in half and put them in the jar (don't add the whole amount at once; add time to time with salt and squeeze them with a pestle)
- Once the jar is filled, place the plastic bag with water, the lid and the airlock
- Keep the jar in a dark cupboard at room temperature

**The procedure was made in double

II: Cherry tomatoes with cabbage leaves on the top

Ingredients:

- 1kg of cherry tomatoes
- 30 grams of sea salt
- 1 cabbage

Procedure:

- Wash and cut the tomatoes in half and put them in the jar (don't add the whole amount at once; add time to time with salt and squeeze them with a pestle)

- Once the jar is filled, place the shaped cabbage leaves on the top , the lid and the airlock
- Keep the jar in a dark cupboard at room temperature

**The procedure was made in double

d. Cucumber

I: Spontaneous vs. induced fermentation

Ingredients:

- 4 cucumbers per jar
- ½ onion cut in rings
- 2 tablespoon of fresh dill (if isn't fresh add only ½ tablespoon)
- 2% sea salt; starter cultures

Procedure:

- Wash and slice the cucumbers
- Cut the half onion in rings and add place all the ingredients in a bowl
- Add the dill and mix everything
- Place the content in the jar and add the brine with the correspondent starter culture
- Shuffle all the content very well and place shaped cabbage leaves on the top
- Close the jar by the lid and the airlock
- Keep the jars in a dark cupboard at room temperature

Note: As the cucumbers lose liquid when sliced, not necessary to add brine, only 5 table-spoons of the cucumbers water have been removed and replaced by 5 tablespoons of brine.

e. Bell pepper

I: Induced vs. spontaneous fermentation

Ingredients:

- 4/5 paprika per jar
- 40 grams of sea salt and starter cultures (paprika, rye, cutting edge, cheese whey)
- 1 tablespoon of fresh thyme (if dry add only ½ tablespoon)

Procedure:

- Prepare the brine by dissolving 40 grams of sea salt in 1L of boiled water
- Wash and cut the paprika in small squares and place them into the jar
- Add the thyme and the required brine and mix very well
- Place the lid on the jar and the airlock
- Keep the jars in a dark cupboard at room temperature

II: Blanched vs non-blanched backslopping inoculation

Ingredients:

- 4/5 paprika per jar
- 40 grams of sea salt and starter cultures (paprika backslopping starter culture)
- 1 tablespoon of fresh thyme (if dry add only ½ tablespoon)

Procedure:

- Prepare the brine by dissolving 40 grams of sea salt in 1L boiled water
- Wash and cut the paprika in small squares and place them into the jar
- Add the thyme, the required brine in proportion 1:1 and a tablespoon of the brine from a previous paprika fermentation (backslopping)
- Place the lid on the jar and the airlock
- Keep the jars in a dark cupboard at room temperature

f. Spent grain

I: Induced vs. spontaneous fermentation

Ingredients:

- 500 gr. fresh spent grain
- Boiled water
- Sea salt and the starter cultures (cutting edge starter culture)

Procedure:

- Add 500 grams of spent grain in the jar
- Prepare the brine at 2% (mix 20 grams of sea salt in 1L of boiled water)
- Add the brine, place the lid on the jar and the airlock
- Keep the jars in a dark cupboard at room temperature

Note: For induced fermentation add the required starter culture to the brine mentioned above and mix it very well before adding to the jar.

g. Measurements

pH:

After 3 days of fermentation, the first pH measure is taken with the pH-meter Testo set 205 (figure 12).Therefore, a certain amount of brine is taken from the jar under sterile conditions. This is repeated every few days when the fermentation is in the fridge in order to check if the fermented product stays stable and for how long.

Note: All the measurements are taken at room temperature (18-20ºC). For all the recipes the initial pH of the brine was measured as well (Day 0 in the pH graphs results).

Figure 12: pH-meter testo 205

Visual inspection:

Every day (including the day of preparation), a visual inspection is done. In this test, 3 parameters are verified:

- If there are the typical CO2 bubbles which indicates that the product is fermenting.
- If the product still stays under the brine and has not floated up
- If there are yeasts or moulds visible

Taste:

If pH and visual inspection are good, a tasting test has been carried out to achieve more information about taste, smell, texture, preferences, and suggestions.

Microbiology:

The following microbiology testes were carried out in order to assure the food safety of the end products :

- total aerobic count (PCA)
- total anaerobic count (RCA)
- Yeasts and moulds (YGC)
- *Listeria monocytogenes*
- *Salmonella sp.*
- *S. aureus*
- *Bacillus cereus*

Note: All microbiology tests were done in double for each sample.

Parameter	Target	Tolerance	Use by date / Best before date
Aerobic (psychrotrophic) count Lactic acid bacteria	Not applicable/not relevant for fermented foods given the use of starter cultures or natural ferments being present. For acidified foods, the aerobic count and lactic acid bacteria might be variable. If being used as a process hygiene indicator, threshold values need to be set in-house, case by case, by baseline studies		
Yeasts (a)	3×10^3	3×10^4	3×10^5
Moulds (a)	3×10^2	3×10^3	No visible mould formation
E. coli	< 10	100	1×10^2 3×10^3 (b)
coagulase-positive staphylococci	3×10^2	3×10^3	3×10^3 1×10^5 (b)
Sulfite reducing clostridia	3×10^2	3×10^3	3×10^5
presumptive *Bacillus cereus*	3×10^2	3×10^3	3×10^3 1×10^5 (b)
Salmonella spp.	Absence in 25 g		
Listeria monocytogenes [1]	**Absence in 25 g (c)**	Absence in x g (c)(d) or **< 100/g (e)**	**100**

Table 3: EU legal criteria for fermented or acidified (pickled) fruit and vegetable products. The results are expressed in cfu/g (colony forming units per gram) (a): Not applicable if yeasts or moulds are part of the functional microbiota Source: Microbiological guidelines: Support for interpretation of microbiological tests results of foods, February 2018.

3.4 Results

a. Cauliflower

I: Optimal salt concentration spontaneous fermentation

Visual inspection:

Every day, there was a visual inspection As the results weren't been positive, there wasn't any tasting test.

The experiment shows the behaviour of cauliflower at different salt concentrations (4%, 10% and 16%). See figure 13, next page. The jar content at 4% turned quite fast from clear to turbid. Until day 4 the smell of the jars remained good.

Secondly a change in colour appeared on the cauliflower at 10%. On day 9, the smell of both jars (4% and 10%), presented a typical cabbage fermentation smell, being stronger on the jar of 4%.

On day 12 (image 3), the colour of the jar content at 16% also changed from clear to turbid and on day 18 started to have a fermented cabbage smell as well. On day 19, the jar at 10% got spoiled and on day 27 the jar at 16% was spoiled.

It seems that the more salt you add, the more microorganism are inhibited.

Figure 13: Spontaneous fermentation of the cauliflower using different concentrations of salt (4%, 10%, 16%). The black number indicates the day of the fermentation. The jars haven been at room temperature during the twenty-seven days that the tests were carried out.

pH:

The initial pH of the cauliflower was 6,05 and the pH of brine was 8,41. Measured at two days of fermentation the pH of cauliflower at 4%, 10% and 16% decreased to 5,86, 5, 85, and 5,6 respectively (figure 14, day 0 and 2). The next two days the pH continued to drop but remaining too high for fermented vegetables. From 7th day to 22th day of the fermentation the pH increased instead of decreasing, being the cauliflower at 4 % the one that presented the highest pH value.

The pH remained too high during the whole fermentation process.

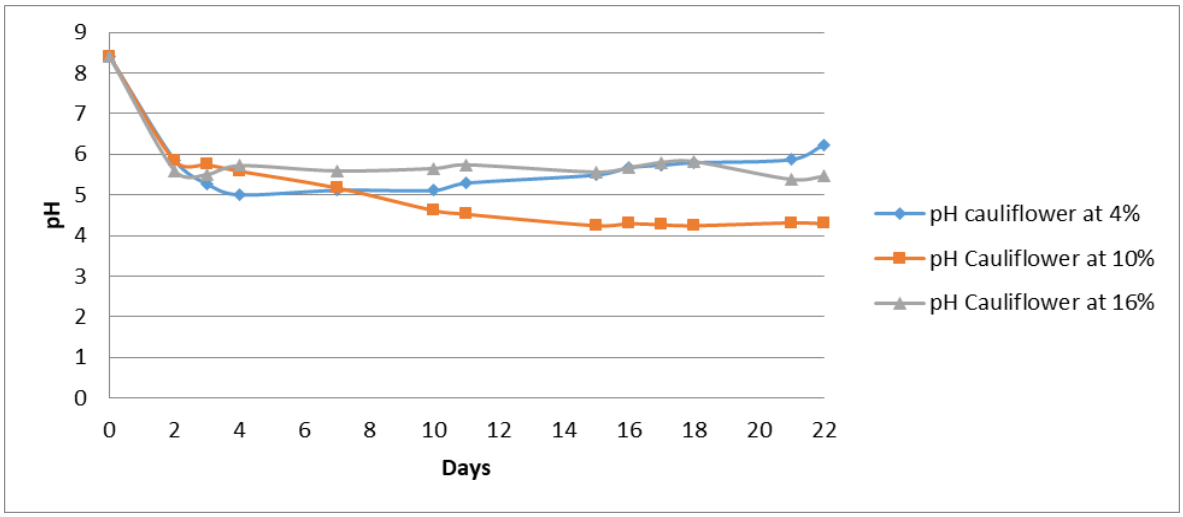

Figure 14: pH results of the fermented cauliflower at different concentration of salt. The initial pH corresponds to the brine used for each jar.

Microbiology:

Figures 15 to 21 show the microbial evolution of the fermented cauliflower at different concentrations of salt. Regarding the aerobic, anaerobic and lactic acid bacteria, it has an important growth on the third day of the fermentation process, being the cauliflower at 4% the one that presented a higher growth comparing with the 10% and 16%. Regarding *Escherichia coli* and sulphate reducing clostridia, they weren't detected during the 22 days of the experiment.

This results show that the NaCl concentration has an important impact on the microbial growth. The salt concentration causes changes in bacterial growth because of the osmotic balance required for bacterial growth. Some microorganisms require an astonishingly high level of salt to begin growth (halophilic), whereas others would immediately be killed at such high salt levels (J. Bautista-Gallego et al., 2013).

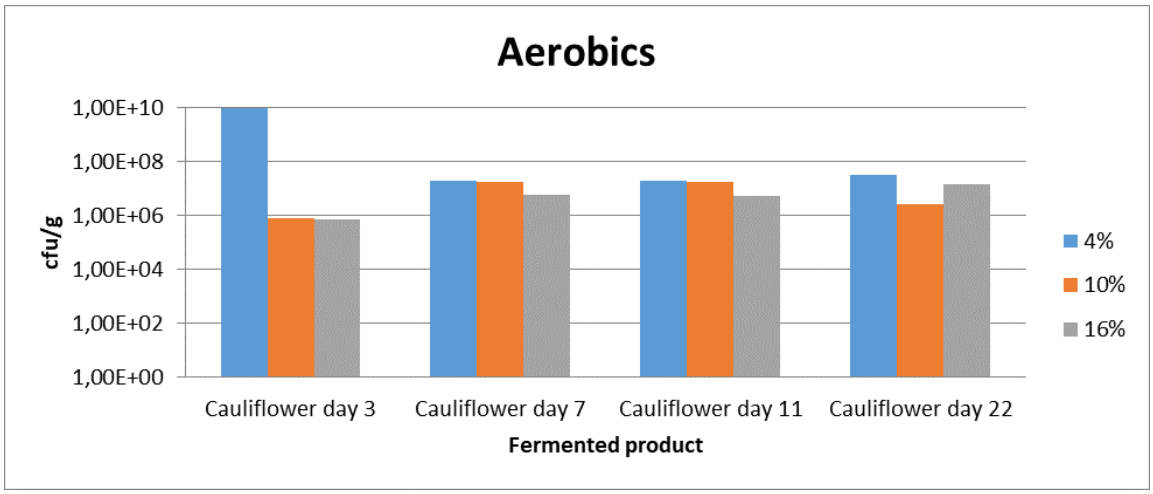

Figure 15: Aerobic bacteria results of fermented cauliflower using aerobic bacteria different concentrations of salt

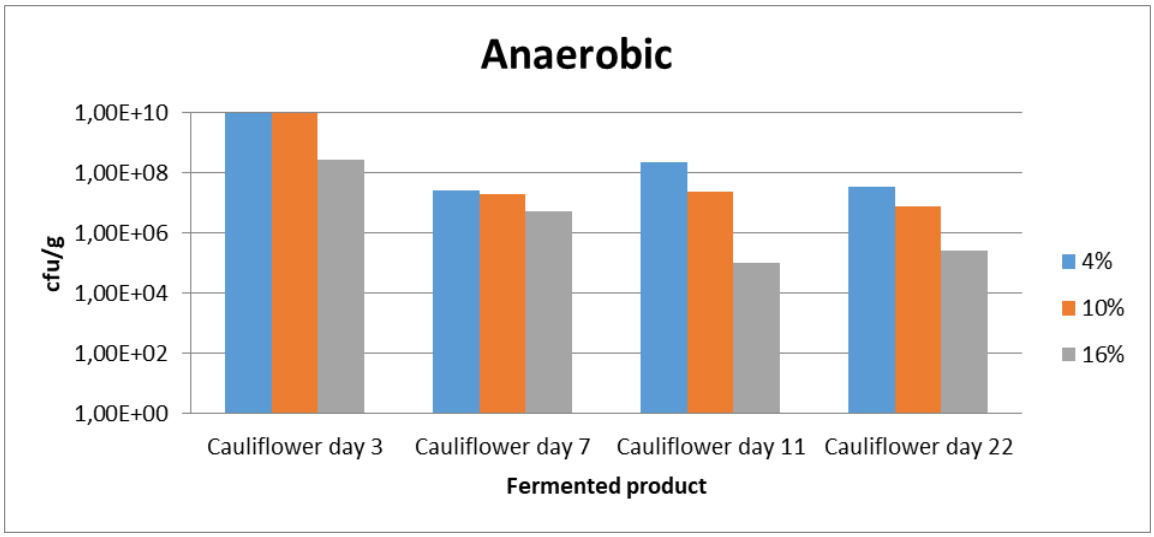

Figure 16: Anaerobic bacteria results of fermented cauliflower using different concentration of salt

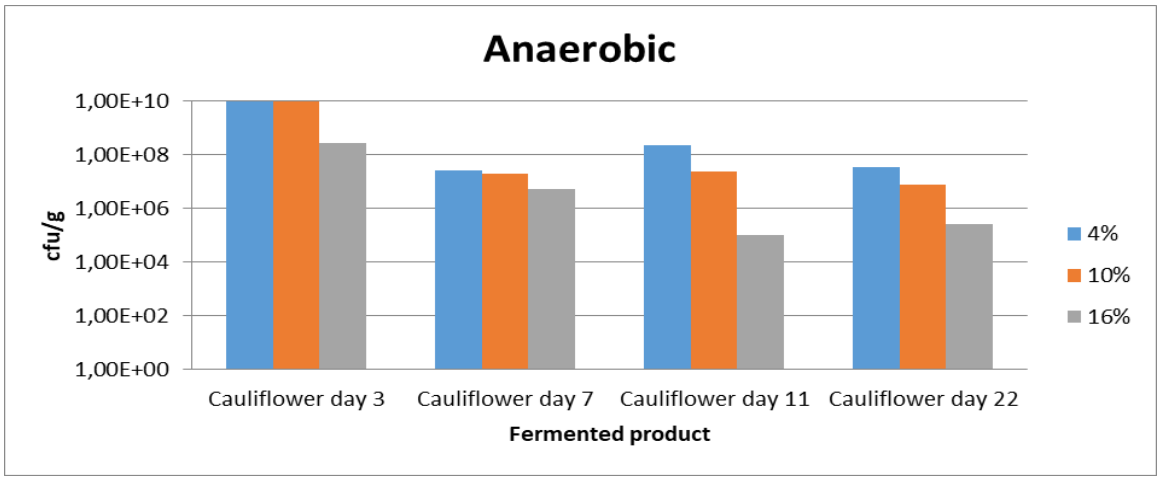

Figure 17:: Lactic acid bacteria results of fermented cauliflower using different concentration of salt

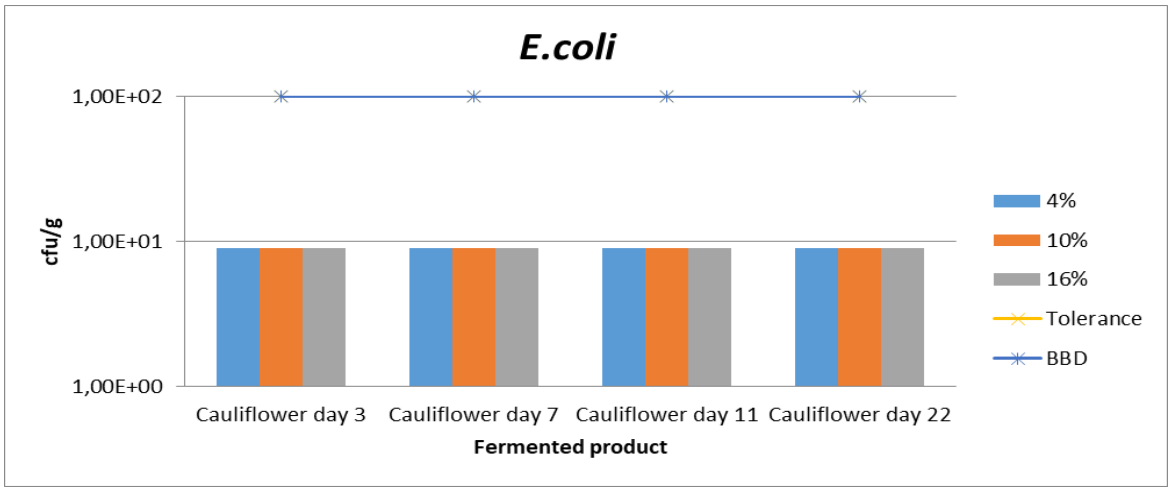

Figure 18: E. coli results of fermented cauliflower using different concentrations of salt

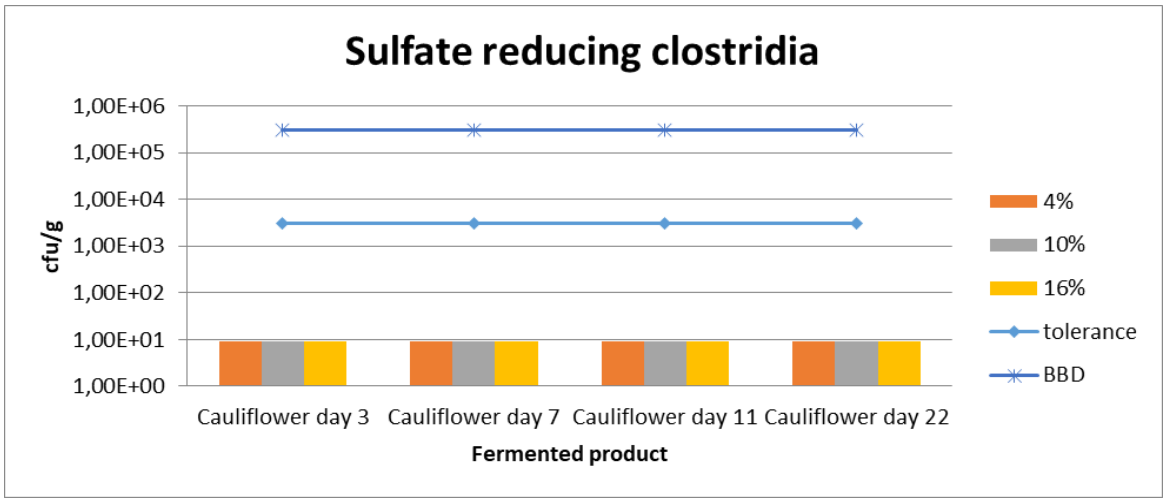

Figure 19: Sulphate reducing clostridia results of fermented cauliflower using different concentrations of salt

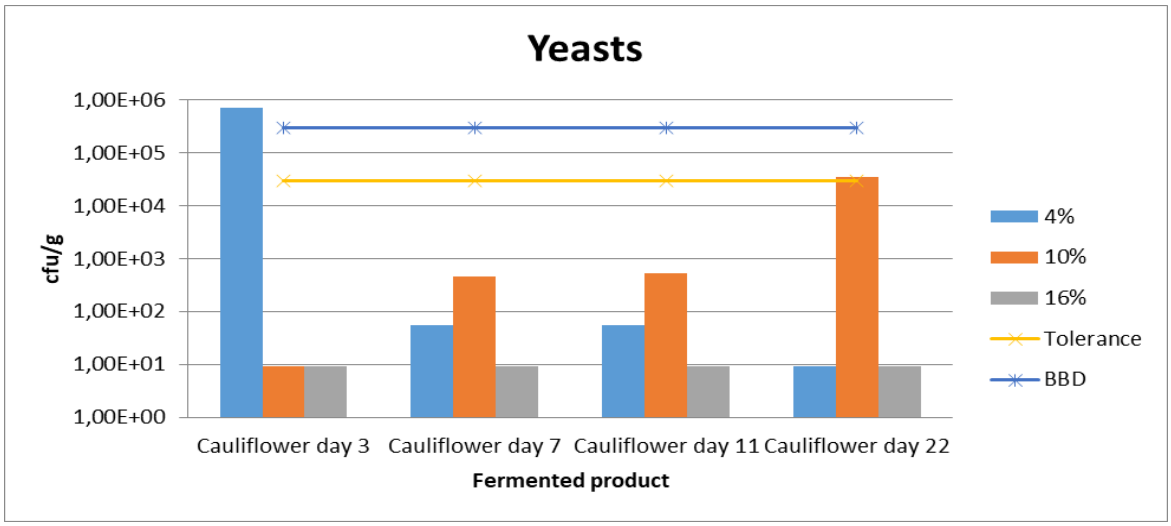

Figure 20: Yeasts results of fermented cauliflower using different concentrations of salt

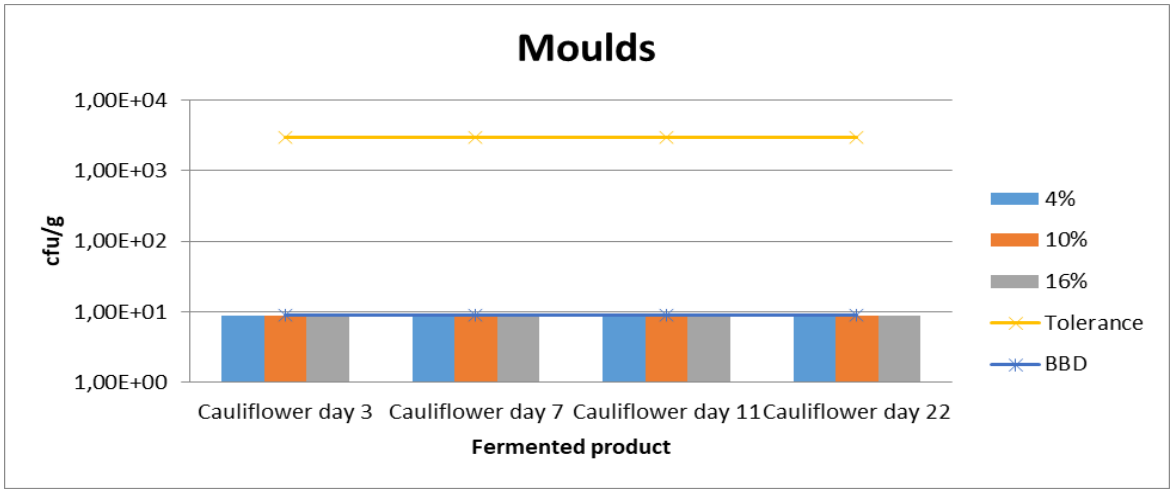

Figure 21: Moulds results of fermented cauliflower using different concentrations of salt

Discussion and conclusions:

Naturally occurring microbial populations in raw materials as well as environmental conditions, such as pH, temperature and salt concentration, determine the outcome of spontaneous vegetable fermentations. Gram-negative aerobic bacteria and yeasts dominate the microbiota of fresh vegetables with lactic acid bacteria constituting a minor, and very often undetectable, portion of the initial population. This was also the case in the present study. Cauliflower on 4% NaCl brine was dominated by other bacteria and yeasts while lactic acid bacteria were minor. Cauliflower at 10% NaCl and 16% NaCl brine inhibited the yeasts and moulds but perhaps, other kind of bacteria has grown and this affected the final product. Some authors revealed that the surface microbiota of the cauliflower consists of Enterobacteriaceae, coliforms and yeasts/moulds. More accurately, the total aerobic mesophilic count is 4.22 cfu/g. Enterobacteriaceae, coliforms and yeasts/moulds are enumerated at 3.50 and 3.82, 2.44 cfu/g (Spiros Paramithiotis et al., 2012).

The pH drop that happened in the first days can be explained by the fact that the microorganisms started to use the fermentable sugars. The fact that the pH did not continue to drop, may be explained by the microbiota competitiveness. As the pH wasn't low enough, it allowed unwanted microbiota to grow and to compete with the LAB. As a result the spontaneous fermentation of this experiment wasn't successful.

As we didn't obtain the whished pH drop, no official tasting tests have been done. To check the effect of the different salt concentrations on the taste of the fermented product, a new fermentation test with cauliflower has been performed and has been tasted at day 3 of the fermentation test (food save fermented product). These sensory tests made it clear that only fermentation with a salt concentration of about 4% is acceptable for the taste. Salt concentrations of 10% and 16% were way to salty. Therefore, only salt concentrations about 4% will be used in the following tests.

It is important to remark that the raw material quality is very important in order to achieve a good spontaneous fermentation. If the raw material present illness or is too old a blanching process might be needed, but then the fermentation has to be induced by a starter culture.

II. Induced vs. spontaneous fermentation

For the induced fermentation, different starter cultures (rye, oral prebiotic, kefir bioferment, backslopping from a spontaneous fermentation of paprika) were compared with the spontaneous fermentation using a 4% NaCl brine solution (10% and 16% give a too salty taste). H.P Flemming and R.F McFeeters claimed that if the vegetables are properly brined, concentrations of up to about 8% are enough to inhibit unwanted microorganisms. Another purpose of the experiment was to check if there is an influence when plastic bags or cabbages leaves are used in order to keep the cauliflowers under the brine.

Visual results:

The present study shows a comparison between spontaneous and induced fermentation of the cauliflower during 8 days (see figures 22 to 26). There is no significant difference between both types of fermentation. Only a little difference between the different starter cultures (figure 23), being the kefir and rye the ones who give a sour end product. It occurs because the rye starter culture, as mentioned in the literature, contains yeasts besides the LAB and the kefir is used more to fermented dairy products, which has, depending on the product, a sour taste. Regarding the colour and general appearance, both maintained good even after 46 days of fermentation.

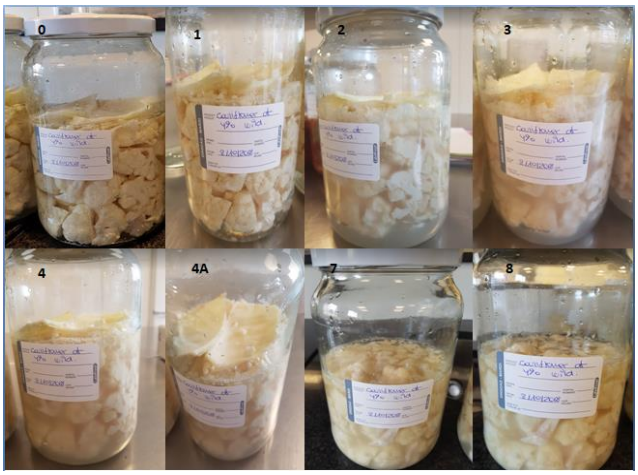

Figure 22: Spontaneous fermentation of the cauliflower at 4% during 8 days , using cabbage leaves on the top in order to keep the cauliflower submerged under the brine. The black number indicates the day of the fermentation

Figure 23: Induced fermentation of the cauliflower by the paprika starter culture during 8 days, using cabbage leaves on the top in order to keep the cauliflower submerged under the brine . The black number indicates the day of the fermentation

Figure 24: Induced fermentation of the cauliflower by the kefir bioferment starter culture during 8 days, using cabbage leaves on the top in order to keep the cauliflower submerged under the brine. The black number indicates the day of the fermentation.

Figure 25: Induced fermentation of the cauliflower by the Rye starter culture during 8 days, using cabbage leaves on the top in order to keep the cauliflower submerged under the brine. The black number indicates the day of the fermentation

Figure 26: Induced fermentation of the cauliflower by the oral prebiotic starter culture during 8 days, using cabbage leaves on the top in order to keep the cauliflower submerged under the brine. The black number indicates the day of the fermentation

Detailed results:

Day 2:

CHARACTERISTICS		ORAL PREBIOTIC	KEFIR BIOFERMENT	PAPRIKA	SPONTANEOUS	RYE
Colour brine/ cauliflower		Clear/white	Clear/white	Turbid/white	Turbid/white	Turbid/white
Aroma		Not detected*	Not detected*	Not detected*	Not detected*	Not detected*
Taste		Not detected*	Not detected*	Not detected*	Not detected*	Not detected*
General appearance		Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. The cabbage leaves presented a chemical reaction with a change in colour from white to dark green. Co2 bubbles visible.

Day 4:

CHARACTERISTICS	ORAL PREBIOTIC	KEFIR BIOFERMENT	PAPRIKA	SPONTANEOUS	RYE
Colour brine/ cauliflower	Turbid/white	Turbid/white	Turbid/white	Turbid/white	Turbid/white
Aroma	Good, acidic	Good, acidic	Good, acidic	Good, acidic	Too acid
Taste	Good, similar to cauliflower pickle	Too sour	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Too sour
General appearance	Ok, no yeasts and moulds visible. Co2 bubbles visible. The cabbage leaves maintained a good colour.	Ok, no yeasts and moulds visible. Co2 bubbles visible. The cabbage leaves maintained a good colour.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible. On the top of the cabbage leaves, one piece of cauliflower was dry.	Ok, no yeasts and moulds visible. Co2 bubbles visible. One of the cabbage leaves presented a chemical reaction visible on a side.

Day 7:

CHARACTERISTICS	ORAL PREBIOTIC	KEFIR BIOFERMENT	PAPRIKA	SPONTANEOUS	RYE
Colour brine/ cauliflower	Turbid/white	Turbid/white	Turbid/white	Turbid/white	Turbid/white
Aroma	Good, acidic	A bit too sour	Good, acidic	Good, acidic	Similar to a yeast fermentation
Taste	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Not detected *
General appearance	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible. A white layer on the top, as a yeast growth, was visible (a)*.

Day 8:

CHARACTERISTICS	ORAL PREBIOTIC	KEFIR BIOFERMENT	PAPRIKA	SPONTANEOUS	RYE
Colour brine/ cauliflower	Turbid/white	Turbid/white	Turbid/white	Turbid/white	Turbid/white
Aroma	Good, acidic	A bit too sour	Good, acidic	Good, acidic	Similar to a yeast fermentation
Taste	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Good, similar to cauliflower pickle	Not detected *
General appearance	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible	Ok, no yeasts and moulds visible. Co2 bubbles visible.	Ok, no yeasts and moulds visible. Co2 bubbles visible. A white layer on the top, as a yeast growth, was visible (b)*.

*Table 5: Sensory results of the fermented cauliflower during 8 days of close examination. * The jars weren't opened so the aroma and taste wasn't detected. (a) :The sample was mixed with a disinfected spoon in order to mix the visible white layer from the top with the brine. (b): As the white layer was present, the sample hasn't been tasted.*

pH:

The initial pH of the 4% NaCl, rye starter culture, paprika starter culture, kefir bioferment starter culture and oral prebiotic starter culture brine was 8, 41; 7, 73; 7,65; 8,33; 8,52 respectively. The pH showed an important drop from alkaline to acid pH for all the samples without any significant difference comparing spontaneous and induced fermentation. The pH maintained stable during 46 days of fermentation at fridge temperature, except for the cauliflower with Rye starter culture which presented a slowly increasing pH reaching 4, 3 on day 50 (figure 27).

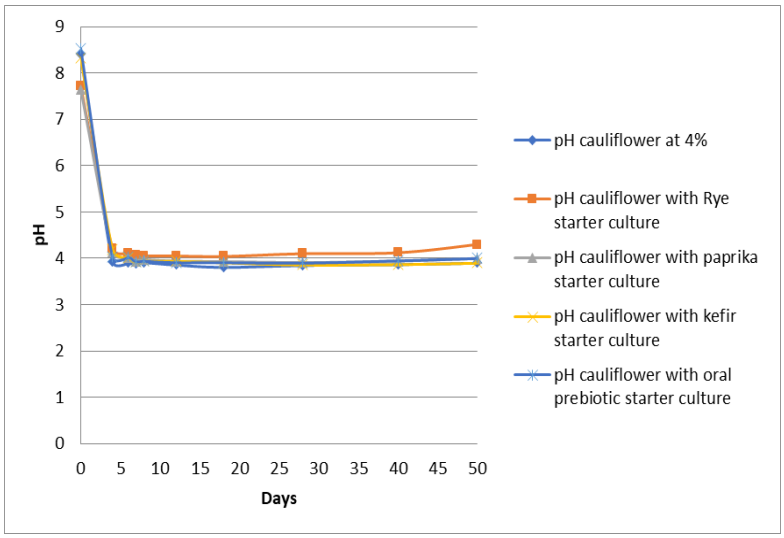

Figure 27: pH evolution of the fermented cauliflower during 50 days of the experiment. Day 0 corresponds to the initial pH of the added brine.

Microbiology:

Figures 28 to 34 show the microbiology results regarding the spontaneous vs. induced fermented cauliflower. The aerobic, anaerobic and lactic acid bacteria are correlated in this way that if the total amount of lactic acid bacteria increase, the number of aerobic and anaerobic bacteria will also increase. The results show that on day 10th of fermentation, the amount of lactic acid bacteria was quite similar between the different samples. The induced fermented cauliflower by backslopping is the one with the highest amount of lactic acid bacteria on day 10 (3,32x108 cfu/g).

The amount of LAB maintained stable during 50 days of fermentation with no significant differences between the spontaneous and induced fermentation, except for the induced fermented cauliflower by Rye starter culture which showed a decrease in the LAB and an increase in aerobic and anaerobic microbial count. These results show that other kind of microorganisms started to grow and competed with the LAB.

Regarding the yeasts and moulds, the sample that showed the highest amount is the one using rye as a starter culture. These results are logic as the yeasts form part of the functional microbiota. However, when rye is used as a starter culture, on day 50, the amount of moulds increased strongly and exceeded “the best before date” acceptable amount of moulds which indicates that the sample is getting spoiled.

E. coli and sulphate reducing clostridia weren’t isolated during the whole experiment. On day 50 no pathogens (*Salmonella, Listeria monocytogenes*, coagulase-positive staphylococci and *Bacillus cereus*) have been detected in the fermented products.

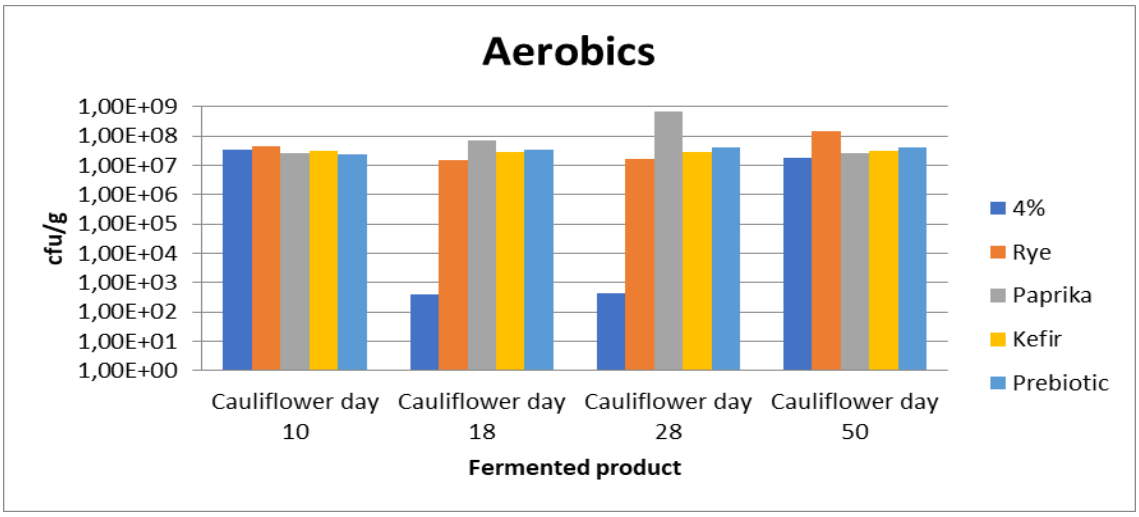

Figure 28: Aerobic bacteria evolution of the fermented cauliflower

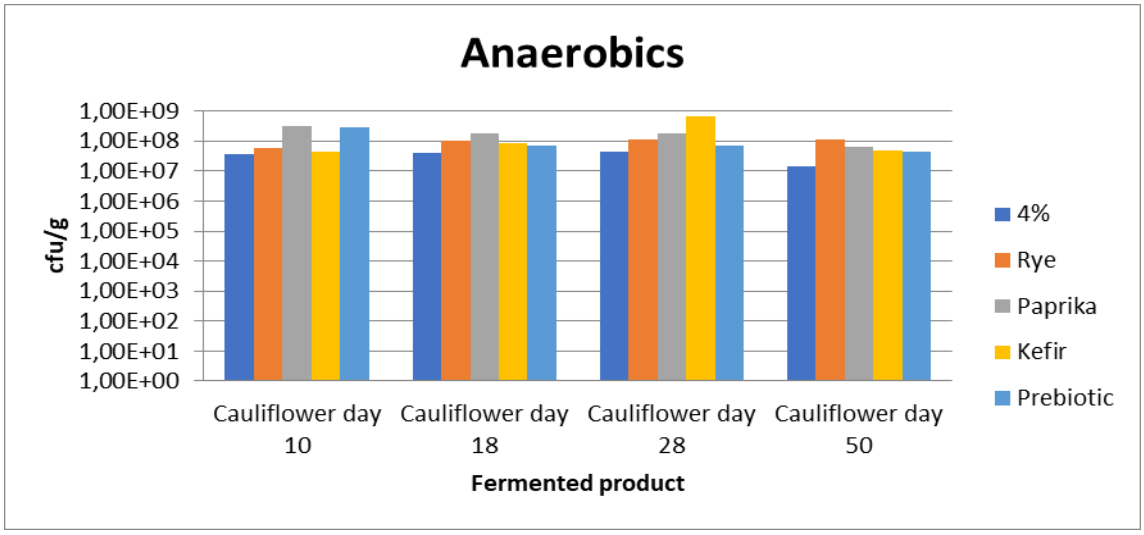

Figure 29: Anaerobic bacteria evolution of the fermented cauliflower

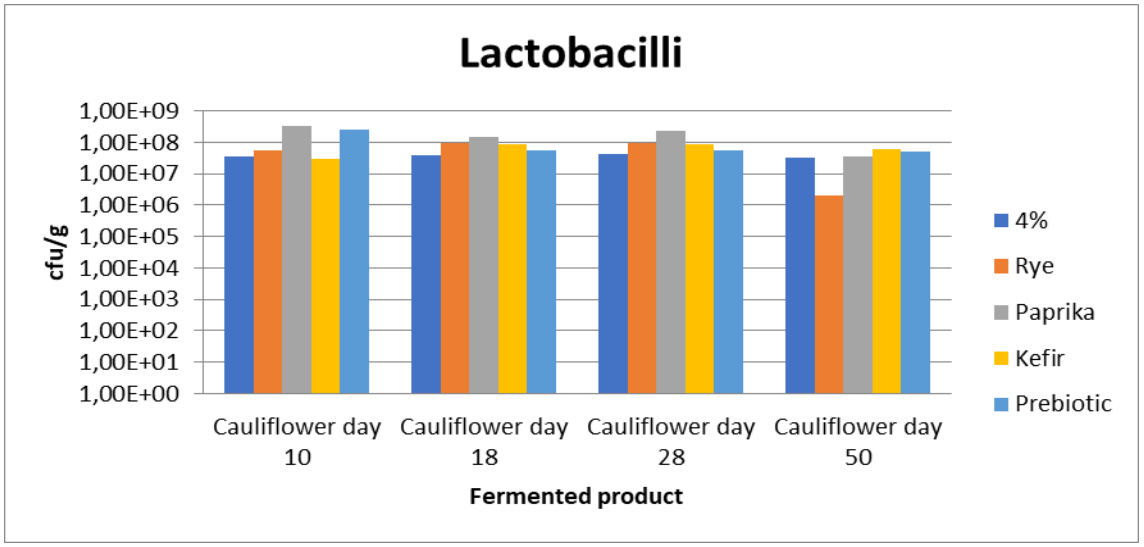

Figure 30: Lactic acid bacteria evolution of the fermented cauliflower

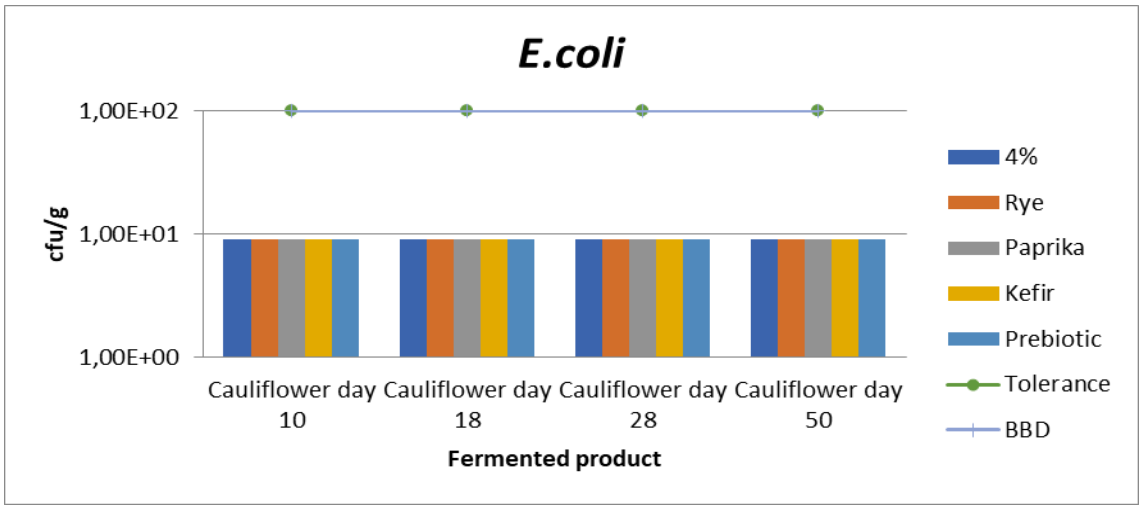

Figure 31: E.coli bacteria evolution of the fermented cauliflower

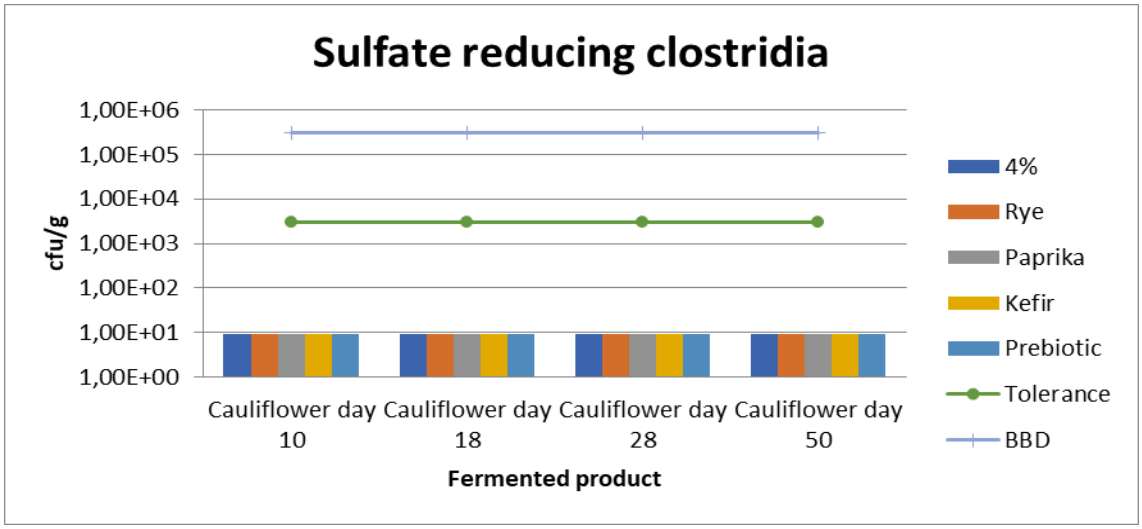

Figure 32: Sulphate reducing clostridia evolution of the fermented cauliflower

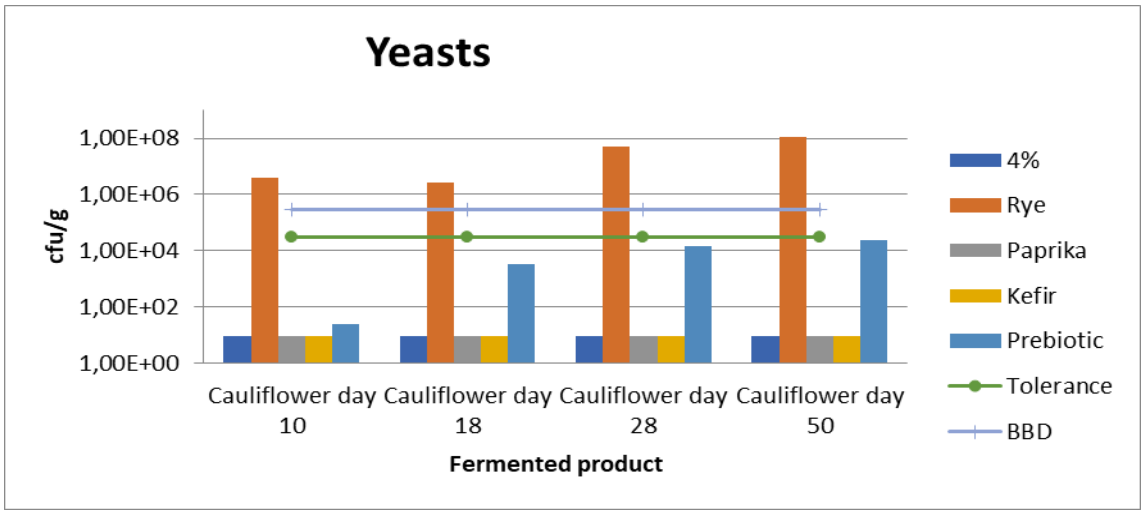

*Figure 33: Yeasts evolution of the fermented cauliflower . ** Rye starter culture has the yeasts as a part of the functional microbiota*

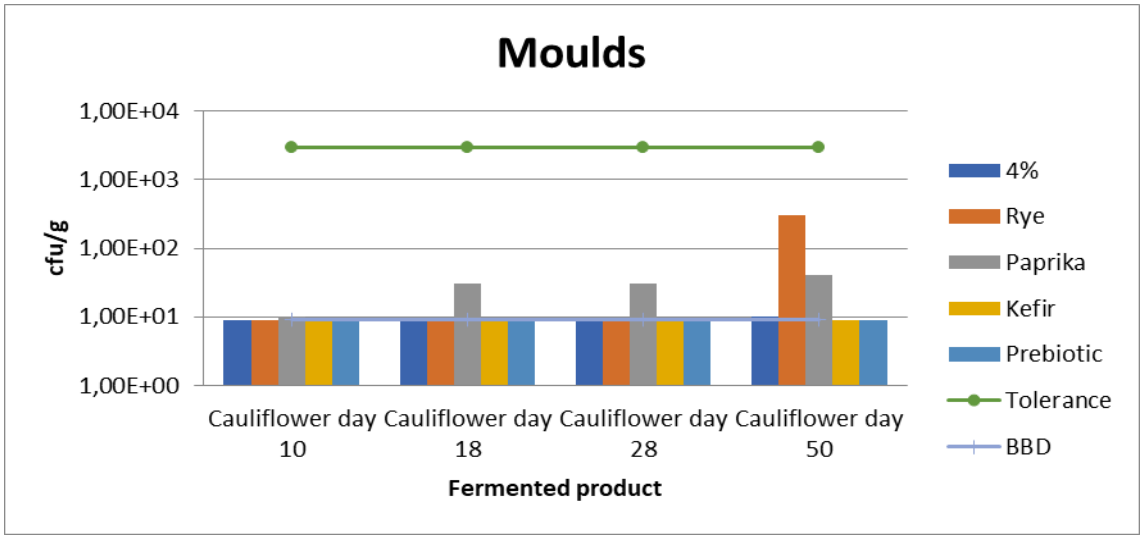

Figure 34: Moulds evolution of the fermented cauliflower

Tasting panel:

A tasting panel was carried out in order to know the judge's opinion and so find out which can be the best option to present the fermented cauliflowers as a final product.

Some pieces from the different jars were taken and dried in the dorex machine (figure 35), at 70ºC during 15 minutes. Figure 36 shows how the samples have been presented to the judges; the numbers have been chosen randomly.

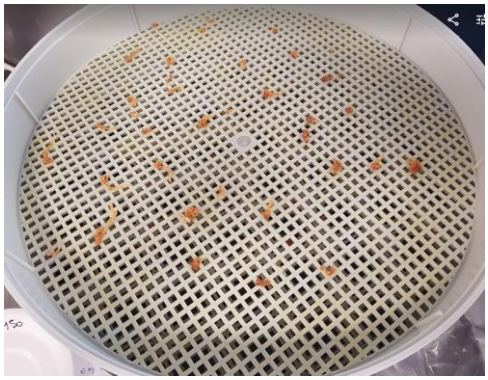

Figure 35: Dorex machine

Figure 36 :The 4 samples of cauliflower that have been presented to the judges(sample 300 corresponds to cauliflower at 4%; sample 350 corresponds to cauliflower with oral prebiotic starter culture; sample 400 corresponds to cauliflower with kefir bioferment starter culture and sample 450 corresponds to cauliflower with paprika starter Culture.

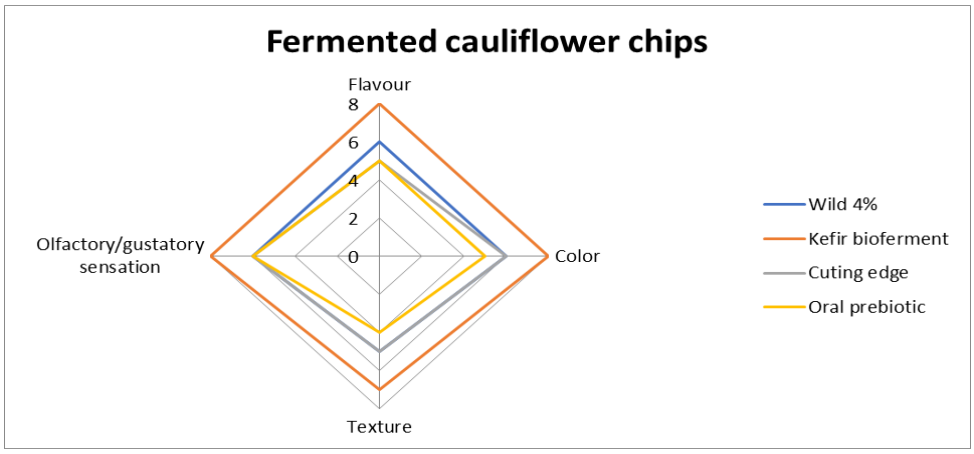

Figure 37: Tasting judges results regarding the fermented cauliflower chips made on the Dorex machine. The scores go from 1 to 9, being 1extremely dislike; 9 extremely like and 5 neither I like nor dislike. In this survey 10 judges participated

Discussion and conclusions:

From this study the following can be concluded:

- The fermentation is a difficult process which inevitably will be leaded by the microbiota of the raw material and of the starter culture used. The environment where the fermentation occurs, the raw material pH and material disinfection are the most decisive parameters whether the fermentation will be successful or not.
- The acceptance of the fermented cauliflower in our culture is not that easy. Most of the judges found that the cauliflowers were too acid, too salty and probably wouldn't eat them as a side dish. This inevitably will lead to use the cauliflowers for other purposes such as the chips. Then the fermenting properties will be lost, because of the high temperatures, but this end product could be used as flavour enhancer or as a substitute of salt in end products such as crackers, soups.. etc.
- Further research will be needed in order to evaluate the fermentation process not only for cauliflower but also for other crops.
- The cabbage leaves don't affect the fermentation but, as the experiment was carried out only once, it can't be concluded yet that cabbage leaves are working better than the plastic bags

b. Tomato

I. Induced vs. spontaneous fermentation

Visual inspection:

On the third day of fermentation a dry layer on the top has been visible due to the formation of two layers. The smell wasn't yet typical of an alcoholic fermentation. From the 6th day on a clear fungal growth has been visible and every day the sample has been mixed with a disinfected spoon. See figure 38.

Figure 38 : Spontaneous fermentation of the cherry tomatoes with cooked garlic cloves during the 8 days of the experiment

pH:

A drop in the pH was observed from 4,36 (pH of the cherry tomatoes themselves) to 3,71 and 3,81 for tomatoes 1 and 2 respectively. The pH of the tomatoes 1 continued decreasing and later on increasing reaching to a final pH on 12th day of 3,85. Instead, the pH of the tomatoes 2 increased reaching on 12th day at 3,95, which is 0,10 higher than the other sample. See figure 39.

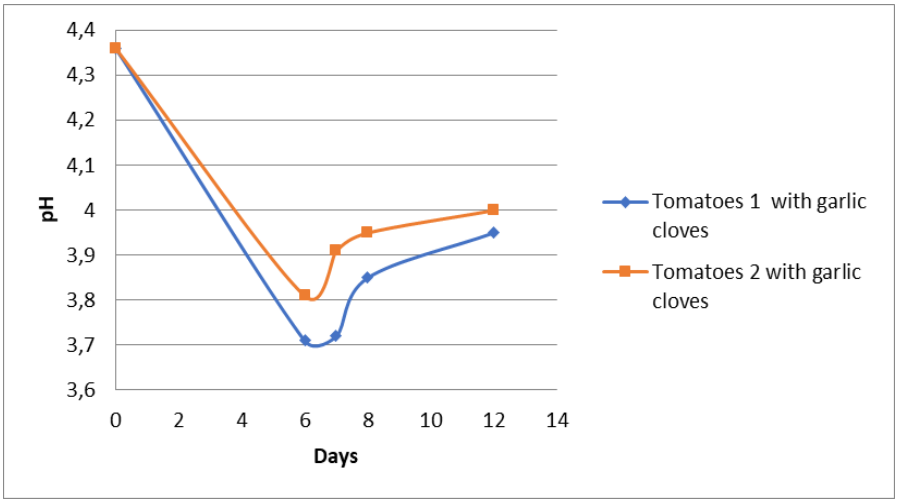

Figure 39: pH results of the fermented cherry tomatoes with cooked garlic cloves during 12 days of experiment

Microbiology

There is no significant difference found in the microbiology between the samples. The aerobic, anaerobic and lactic acid bacteria counts are quite similar. The yeasts count cross the tolerance limit for all the samples, however the moulds have been isolated only on the tomatoes that presented a thicker layer of fungal growth. See figures 40 to 46.

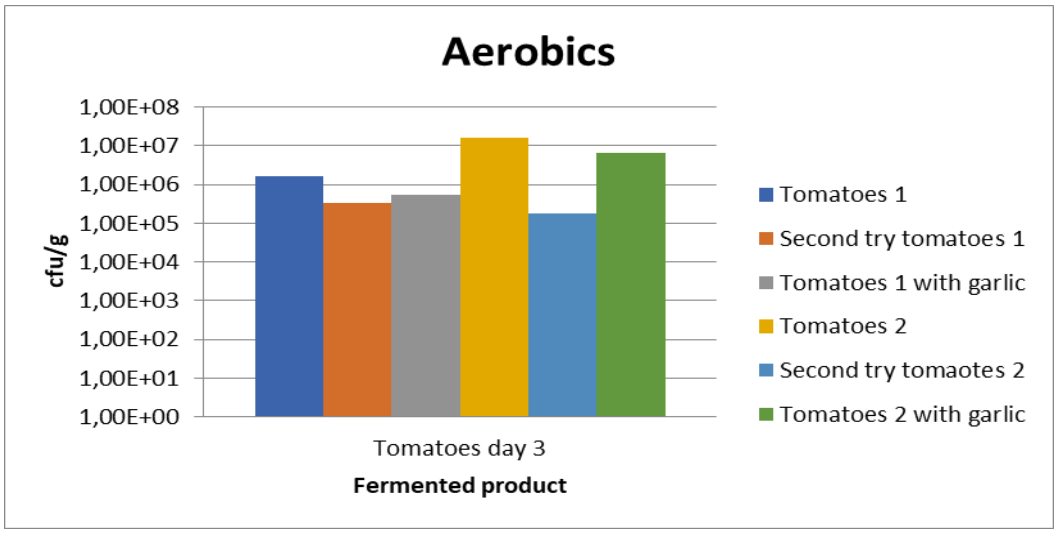

Figure 40: Aerobic bacteria results of the spontaneous fermentation of the cherry tomatoes

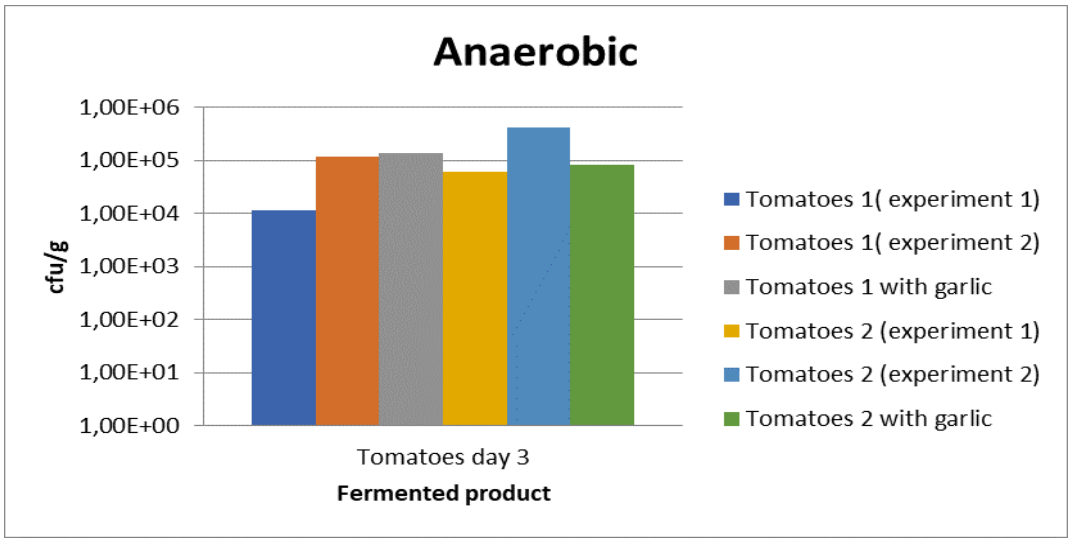

Figure 41: Anaerobic bacteria results of the spontaneous fermentation of the cherry tomatoes

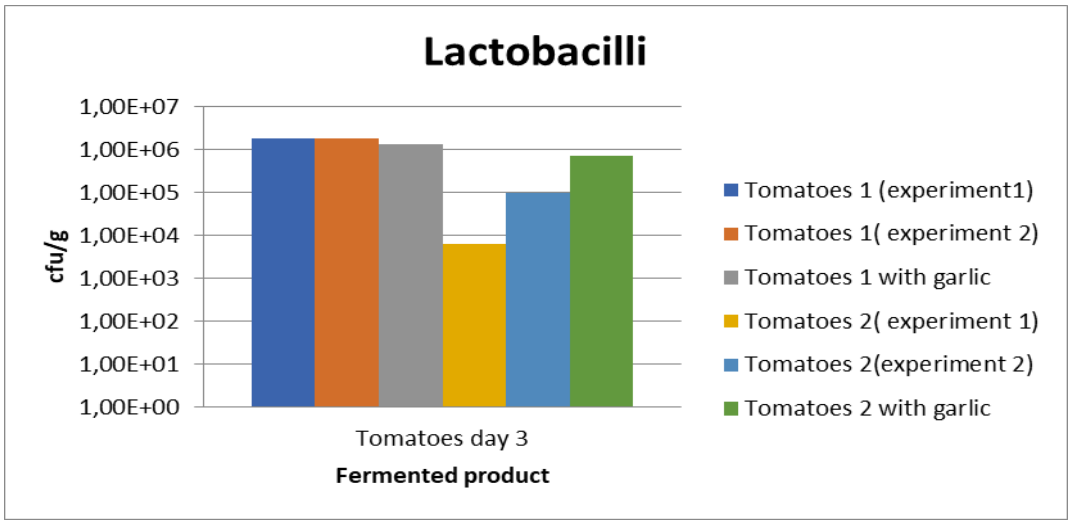

Figure 42: Lactic acid bacteria results of the spontaneous fermentation of the cherry tomatoes

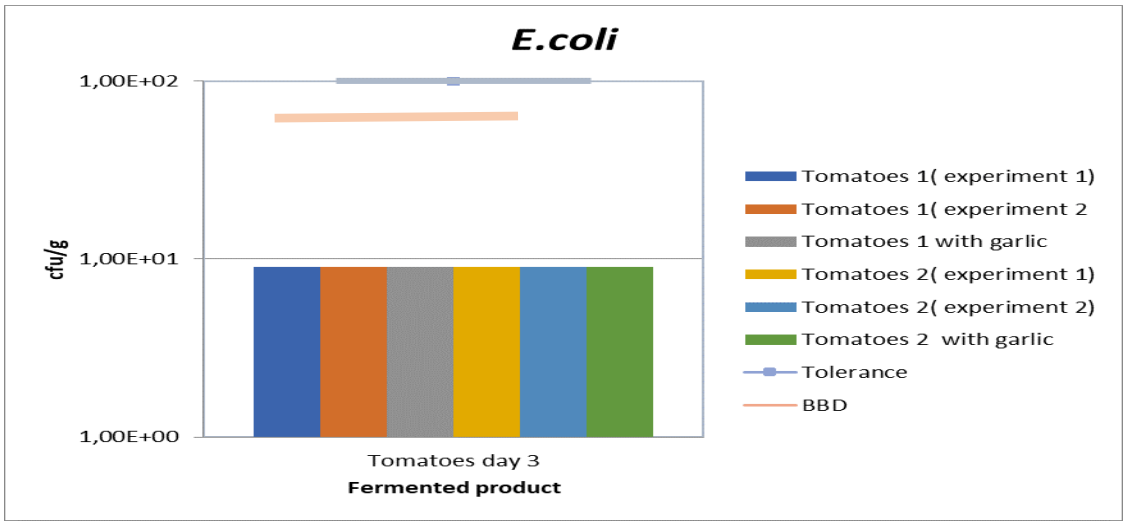

Figure 43: E.coli results of the spontaneous fermentation of the cherry tomatoes

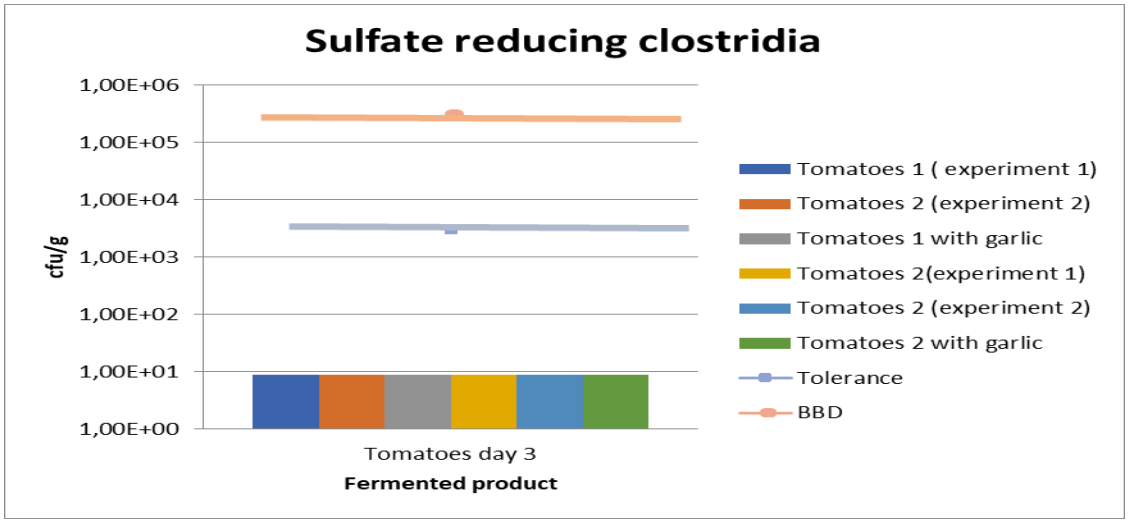

Figure 44: Sulphate reducing clostridia results of the spontaneous fermentation of the cherry tomatoes

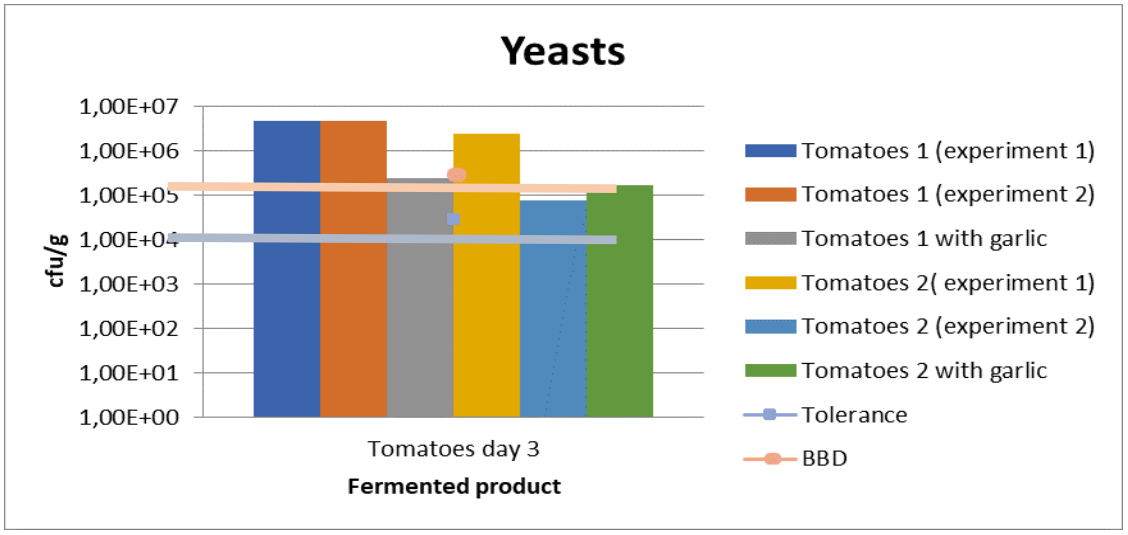

Figure 45: Yeasts results of the spontaneous fermentation of the cherry tomatoes

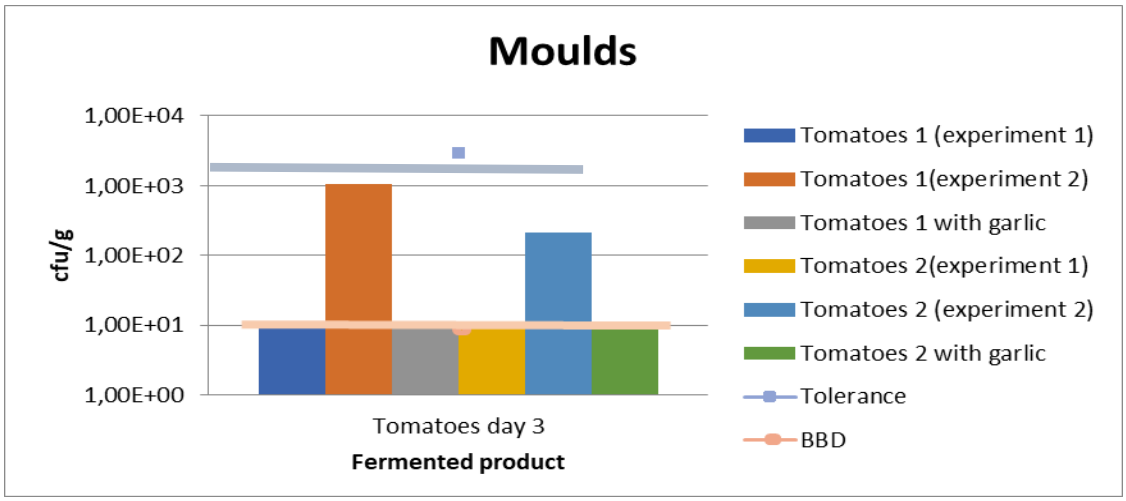

Figure 46: Moulds results of the spontaneous fermentation of the cherry tomatoes

Discussion and conclusions:

Differences in osmotic potential between the fruit and the surrounding soluble solid (NaCl) lead to the generation of two layers, and consequently to a contamination of the product as it was getting dry on the top. However, Pederson (1936), mentions that L. plantarum has been found in fermenting and spoiled tomato products forming pustules because of a massive subepidermal growth. In this study the use of an antifungal (garlic gloves) was studied and the results were the same as without using any antifungal. This might be because of the microbiota of the tomatoes themselves. The cherry tomatoes are a sensitive fruit and the storage and distribution in the market play an important role in food safety of this kind of product. One study reveal that the cherry tomatoes from the supermarket contained coliforms ranging from 1.0 to 1.8 log CFU/g; moulds and yeasts ranging from 2.0 to 4.1 log CFU/g; aerobic bacteria ranged between 2.2 and 4.4 log CFU/g (Leal-Cervantes et al., 2018).

Summarising the results obtained the fermentation of the cherry tomatoes needs further research, perhaps the use of essential antifungal oils or ferment the entire tomatoes in a brine solution of NaCl could help prevent the fungal growth and obtain a good end product.

c. Cucumber

I. Induced vs. spontaneous fermentation

Visual inspection:

On the second day of fermentation, a small layer of liquid appeared on the bottom due to a difference in osmotic potential, but still the cucumbers were submerged in the brine. The third day this layer was bigger and the cucumbers started to get dry on the top. In order to save the sample, the jars have been opened and pressed with a disinfected pestle in order to submerge the cucumbers in the brine. However, on 4th day of fermentation the sample floated up again and the cabbage leaves from the top were about to get fungal growth as there was a sliminess texture on it when removed. After pressing and submerging again the cucumbers in the brine it stood stable for the rest of the days (last check 30/11/2018). Regarding the colour, a change was detected from the 4th day of fermentation turning from clear green to yellowish green (Figure 47, from 4th to 11th day). The taste was the typical taste for fermented cucumbers with a spicy note of the dill. The taste remained good until the 11th day of fermentation.

The results obtained show a small difference in the taste between the different starter cultures, being the kefir and the rye the ones who gives to the end product a sour taste. Regarding the colour, there is no difference comparing spontaneous by induced fermentation and either between the different starter cultures. The differences in the osmotic potential that generates the phase separation affects the general appearance of the product, and every time a dry layer of product on the top was observed, with a disinfected spoon the samples have been mixed.

Figure 47: Fermented cucumbers during 11 days of fermentation (black numbers) . The first 4 days corresponds at room temperature fermentation and from day 4 to 11, fridge temperature fermentation.

Detailed results:

Day 2:

CHARACTERISTICS	KEFIR BIOFERMENT	SPONTANEOUS 2% NaCl BRINE	RYE	CUTTING EDGE
Colour	Clear green	Clear green	Clear green but less intense than other samples	Turbid/white
Aroma	Sweet- acidic	Sweet- acidic	Sweet- sour	Sweet- acidic
Smell	Not detected*	Not detected*	Not detected*	Not detected*
Taste	Not detected*	Not detected*	Not detected*	Not detected*
General appearance	The cabbage leave on the top had a bit of brine. Few Co2 bubbles visible. A small phase separation was visible.	Good, Co2 bubbles visible. The cabbage leave looked ok. .	A phase separation appeared and on the top the sample was getting dry. Few co2 bubbles visible.	A phase separation appeared and the sample emerged and got dry on the top. Co2 bubbles visible.

*Jars not opened

Day 5:

CHARACTERISTICS	KEFIR BIOFERMENT	SPONTANEOUS 2% NaCl BRINE	RYE	CUTTING EDGE
Colour	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Aroma	Sweet- acidic	Sweet- acidic	Sweet- sour	Good, acidic
Smell	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle
Taste	Typical of cucumber pickle with bit more of sourness	Typical of cucumber pickle	Not that typical of a cucumber pickle	Typical of cucumber pickle
General appearance	Good	Good.	Good.	Fungal growth appeared on the cabbage leave. When it was removed the sample was a bit dry.

Day 7:

CHARACTERISTICS	KEFIR BIOFERMENT	SPONTANEOUS 2% NaCl BRINE	RYE	CUTTING EDGE
Colour	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Aroma	Sweet- acidic	Sweet- acidic	Sweet- sour	Good, acidic
Smell	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill.	Typical of cucumber pickle
Taste	Typical of cucumber pickle with a bit more of sourness	Typical of cucumber pickle	Not that typical of a cucumber pickle. Sour.	Typical of cucumber pickle
General appearance	Good	Good.	Good.	A small phase separation was visible, but not affected the general appearance of the product.

Day 9:

CHARACTERISTICS	KEFIR BIOFERMENT	SPONTANEOUS 2% NaCl BRINE	RYE	CUTTING EDGE
Colour	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Aroma	Sweet- acidic	Sweet- acidic	Sweet- sour	Good, acidic
Smell	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill	Typical of cucumber pickle with aromatic dill. Too Sour.	Typical of cucumber pickle
Taste	Typical of cucumber pickle with a bit more of sourness	Typical of cucumber pickle	Too sour	Typical of cucumber pickle
General appearance	Good	Good.	Good.	Good.

pH

The pH dropped from basic to acid within 4 days with no significant difference comparing spontaneous by induced fermentation and maintained stable, with a slight difference between the different starter cultures, during 36 days of fermentation. See figure 48.

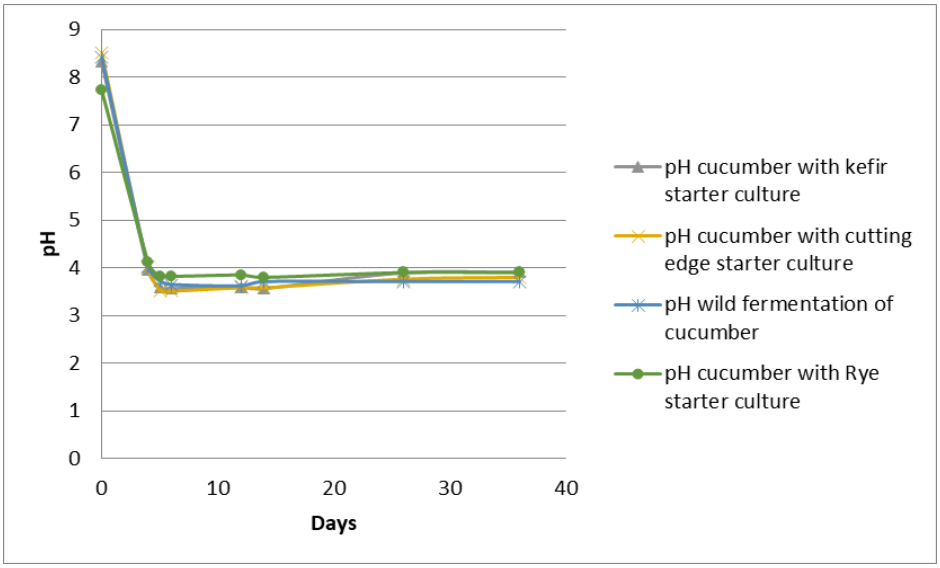

Figure 48: pH evolution during 46 days of experiment. The initial pH corresponds to the added brine

Microbiology:

The results show that there is no significant difference between spontaneous and induced fermentation and either between the different starter cultures. However, the results regarding the yeasts are contradictory and unexpectable as the Rye starter culture (a yeast starter culture), presented lower count comparing to the rest of the starter cultures. Total aerobic and anaerobic bacteria approximated or exceeded the lactic acid bacteria in numbers throughout the fermentation. See figures 49 to 55.

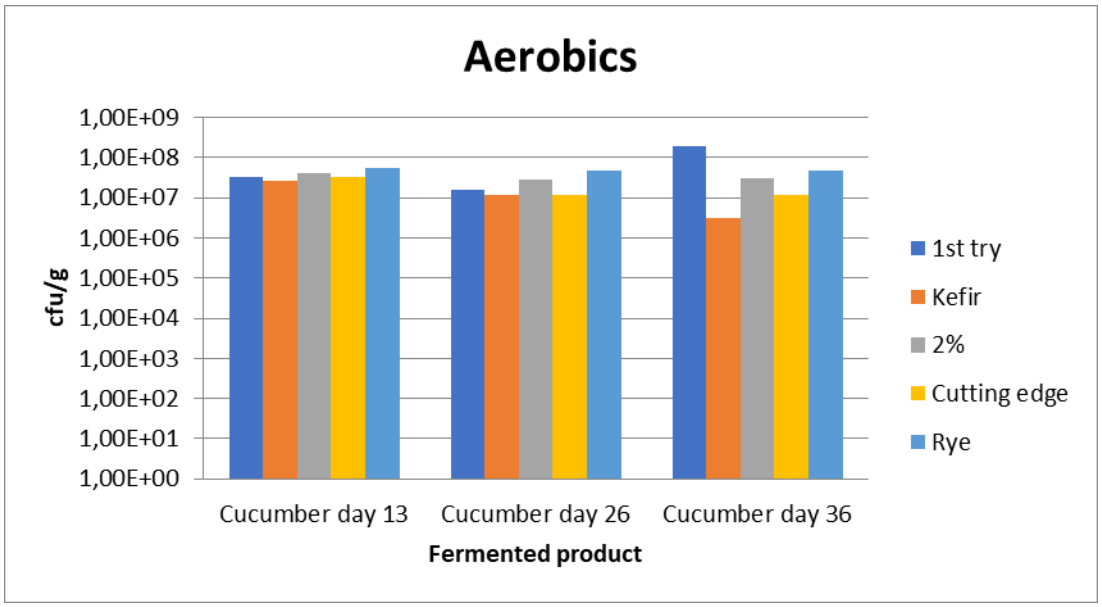

Figure 49: Aerobic plate cont evolution of the fermented cucumbers

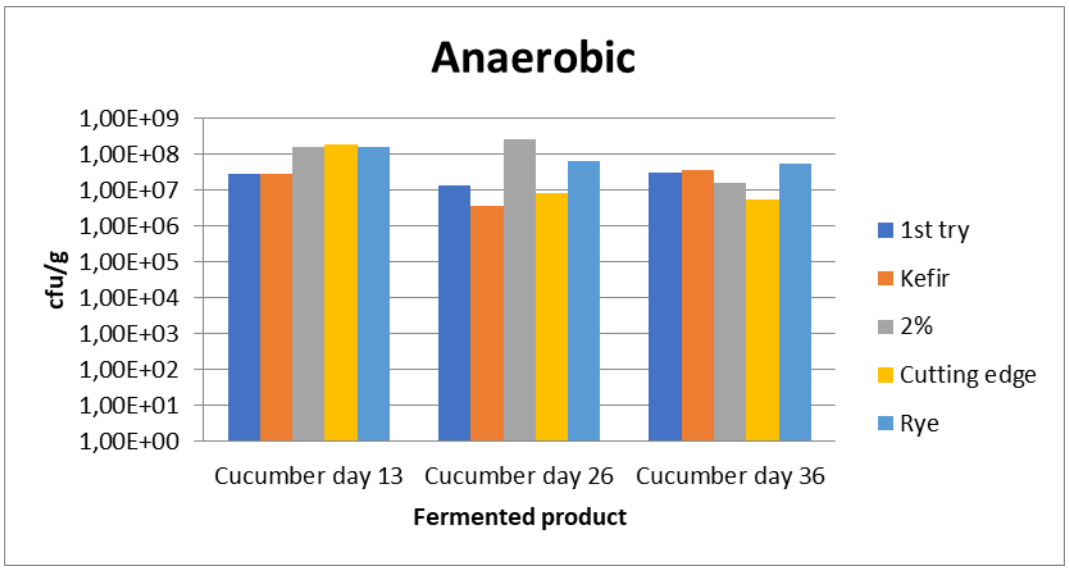

Figure 50: Anaerobic plate cont evolution of the fermented cucumbers

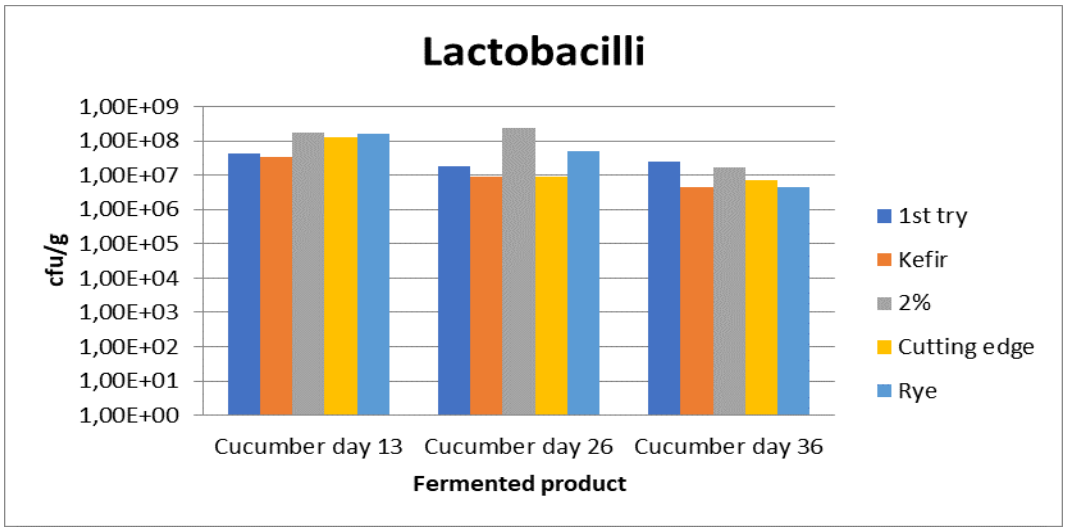

Figure 51: Lactic acid bacteria evolution of the fermented cucumbers

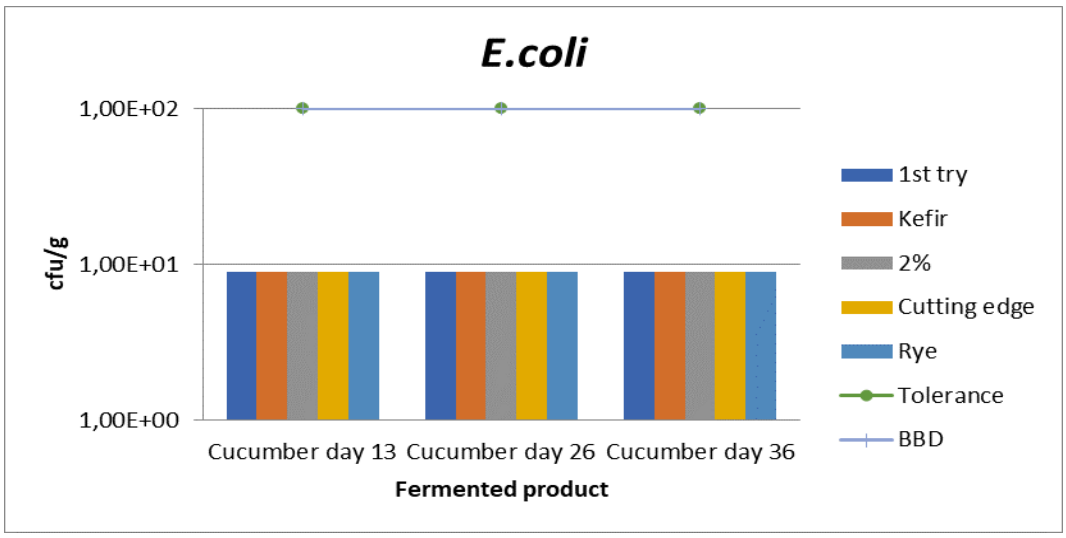

Figure 52: E. coli evolution of the fermented cucumbers

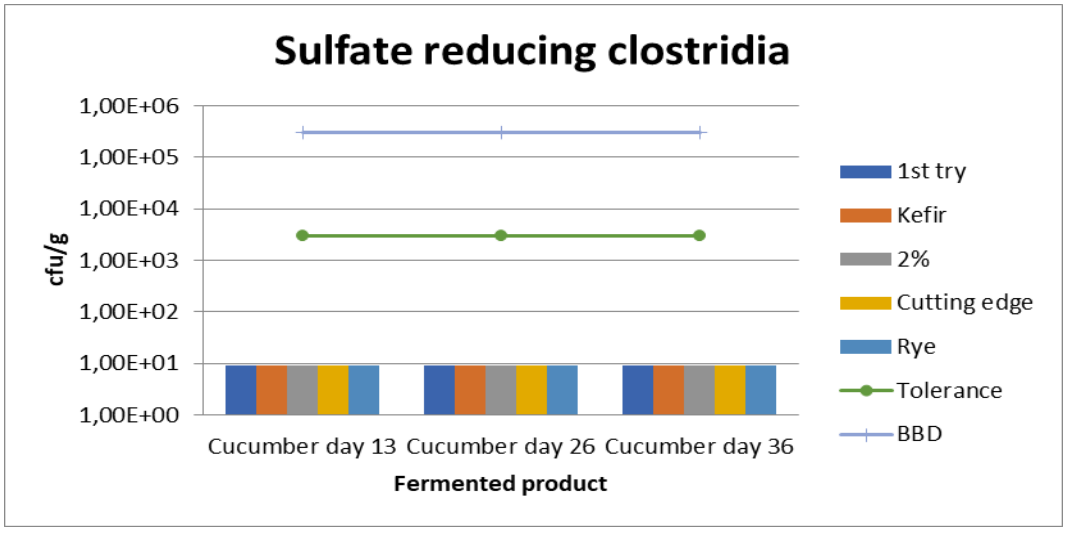

Figure 53: Sulphate reducing clostridia evolution of the fermented cucumbers

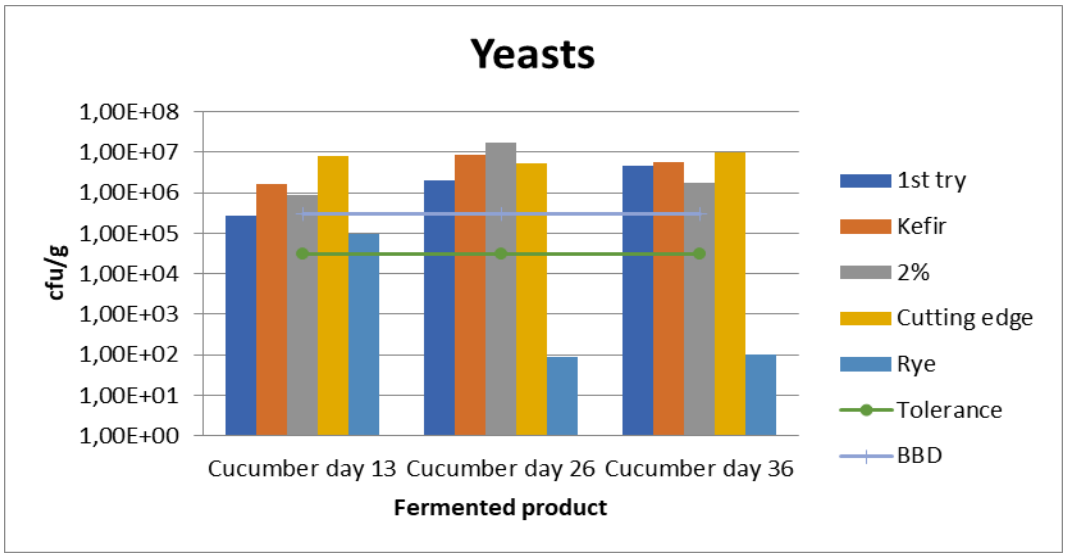

Figure 54: Yeasts evolution of the fermented cucumbers

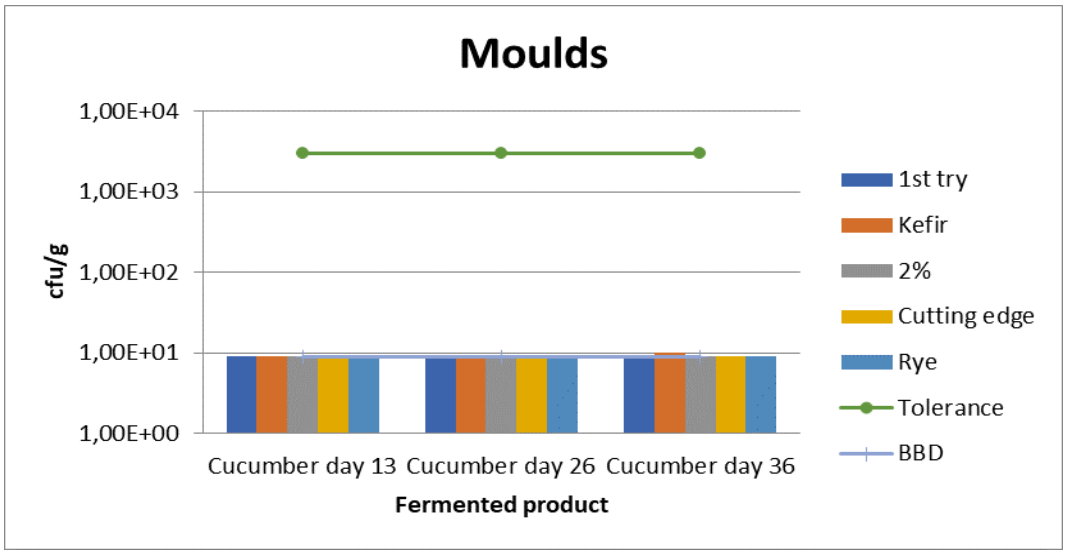

Figure 55: Moulds evolution of the fermented cucumbers

Tasting panel:
Sliced fermented cucumbers were evaluated by a taste panel of 11 people, 2 of whom are used to eat fermented cucumbers, with the purpose of archiving information about taste and mainly texture of the product. See figure 56.

Sliced cucumber fermented in a brine solution of 2% NaCl got the same score for flavour, texture and gustatory/olfactory sensation and less for the colour, being the last parameter the worst evaluated by the panelists in addition with Rye starter culture sample. The sample that the panelists liked more was the one with cutting edge starter culture and the less that the one with Rye starter culture.

Some of the panelists never ate before fermented vegetables, and not liked the texture of the samples; they expected samples with more firmness. The ones that were used with this kind of products not minded the texture but evaluated more the taste and found to sour the sample with Rye starter culture and sour but less intense the one with kefir bioferment starter culture. See figure 57.

Figure 56: The way that the fermented cucumbers have been presented to the panelists

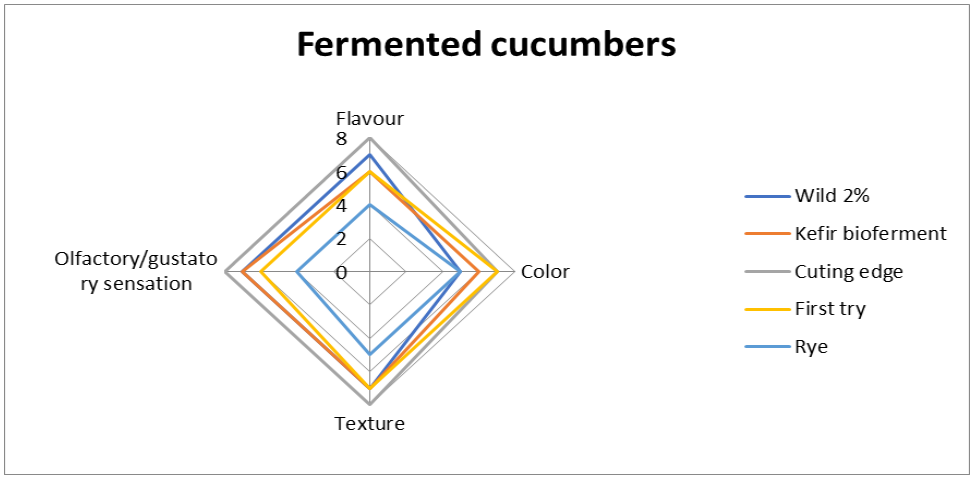

Figure 57: Tasting panel results regarding the sliced fermented cucumbers. The scores goes from 1 to 9, being 1 (extremely dislike) , 5 (neither I like nor dislike) and 9 (extremely like).

Discussion and conclusions:

These results indicate that the cucumbers can be fermented spontaneously and by fermenting starter cultures with no significant difference. When a yeasts starter culture is used the taste and the acceptance of the consumer change. This may be taken into account for a commercial use. For lab scale it was interesting to test the difference that can bring the different starter cultures.

The difficulty of the process is to achieve and keep a good texture of the end product. As the cucumbers are sliced the texture is not firm and so the acceptance may vary according to the taste of each person. Sometimes the samples gave an impression of being spoiled because of the texture and the slightly change in colour. However, the panelists liked the taste of almost all of the samples being the best one the cutting edge starter culture. The microbiology results show a big count of yeasts that haven't been present in the visual inspection that must be investigated as it was expected a bigger count using Rye starter culture.

Summarising the results can be concluded that spontaneous fermentation is as good as induced fermentation. A starter culture more suitable for vegetables gives an end product with better sensory properties. The salt did not help to retain the firmness when the product was sliced.

d. Pepper

I. Induced vs. spontaneous fermentation – small jars

Visual inspection:

The jars presented a massive fungal growth with no significant differences between the samples. See figures 58 to 63.

Figure 58: Spontaneous fermentation at 4% concentration of salt

Figure 59: Induced fermentation of the paprika Rye starter culture

Figure 60: Induced fermentation of the paprika by kefir bioferment starter culture

Figure 61: Induced fermentation of the paprika by paprika starter culture

Figure 62: Induced fermentation of the paprika by the whey starter culture

Figure 63: Induced fermentation of the paprika by the cutting edge starter culture

pH:

The pH had shown an important drop from basic to acid in 5 days of fermentation. It was observed that the induced fermentation helps in the drop of the pH as it dropped faster compared with the spontaneous. However, there are differences in between the different starter cultures used as the initial pH differs. The Rye starter culture presented a slower drop in the drop (from 4, 87 to 3, 52 in 5 days). See figure 64.

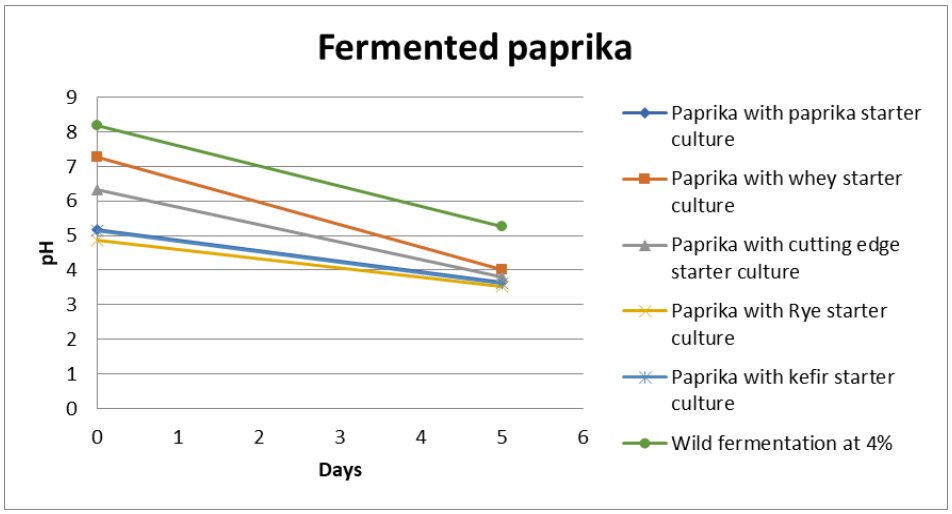

Figure 64: pH of the different samples in the day of preparation and after 5 days of fermentation

II. Induced vs. spontaneous fermentation – normal jars

Visual inspection:

After 4 days of fermentation the samples got spoiled. The separation phase was bigger in the spontaneous fermentation rather than induced fermentation. This can also be due to the amount of paprika added in the jars and the brine as it wasn't the same. See figure 65.

Figure 65: Sensory results of the paprika during 4 days of fermentation. (A) Corresponds to the spontaneous sample and (B) corresponds to the induced sample by cutting edge starter culture.

III. Blanched vs non-blanched paprika backslopping inoculation

Visual inspection:

On third day of fermentation there weren't significant differences between the samples regarding the smell, aroma taste and texture. The smell was found to be typical of a paprika pickle; the aroma was acidic and the texture crispy; taste was found to be sour. However there was a difference in the general appearance doing heating treatment; there were more Co2 bubbles and the colour was bit slightly (figure 65). Without a heating treatment the phase separation was present and the colour of the brine was more turbid.

Figure 66: Sensory results of the fermented paprika by backslapping . The numbers indicated in black correspond to the fermentation day, being A: blanched vs. Not blanched experiment of 7 days of fermentation; B: Blanched paprika experiment of 3 days; C: Non- blanched paprika experiment of 3 days

pH:

The use of a heating treatment previous to the fermentation shows a faster drop in the pH in 3 days of fermentation compared to the non-blanched sample. However, the difference is not that high and in both cases the pH maintains stable during 7 days of fermentation at fridge temperature. See figure 67 on the next page.

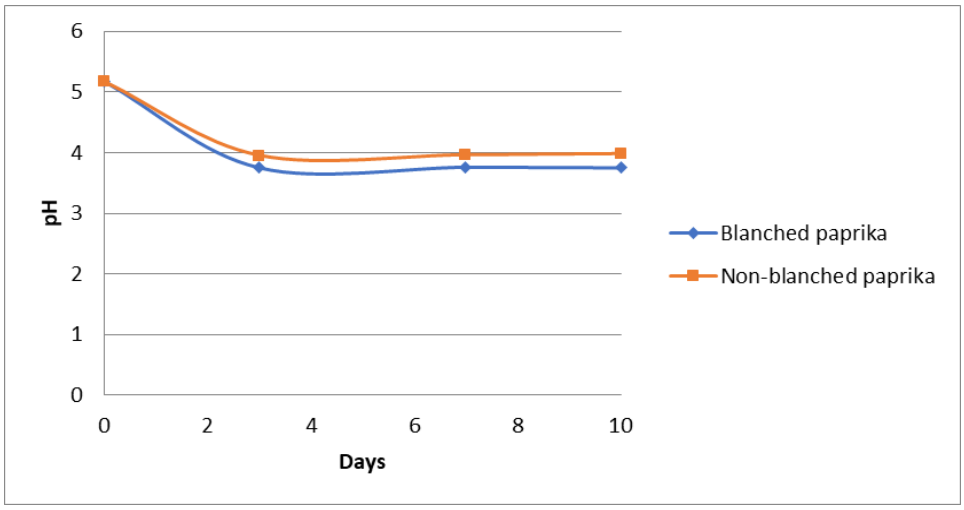

Figure 67: pH evolution comparing a blanch treatment vs. without

Microbiology:

The microbiology results show that there is no significant difference using a heating treatment before fermentation as the microorganisms found are similar in both cases. Regarding the pathogens, Salmonella was absent in 25grams, sulphate reducing clostridia was below the detection limit as well as Listeria monocytogenes, coagulase-positive staphylococci and Bacillus cereus. See figure 68 to 74.

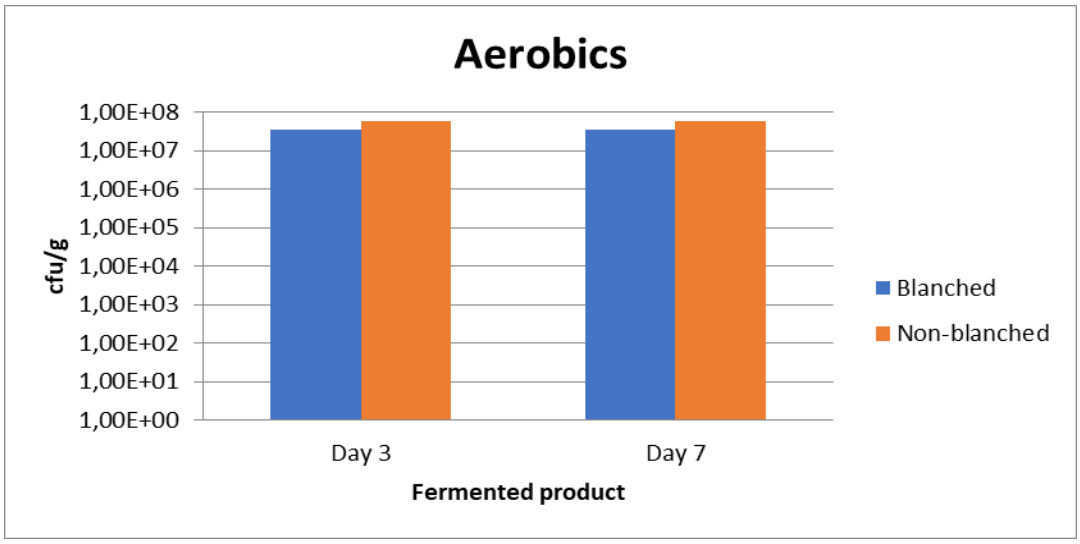

Figure 68: Aerobic bacteria counts of the fermented paprika comparing a heat treatment vs. without

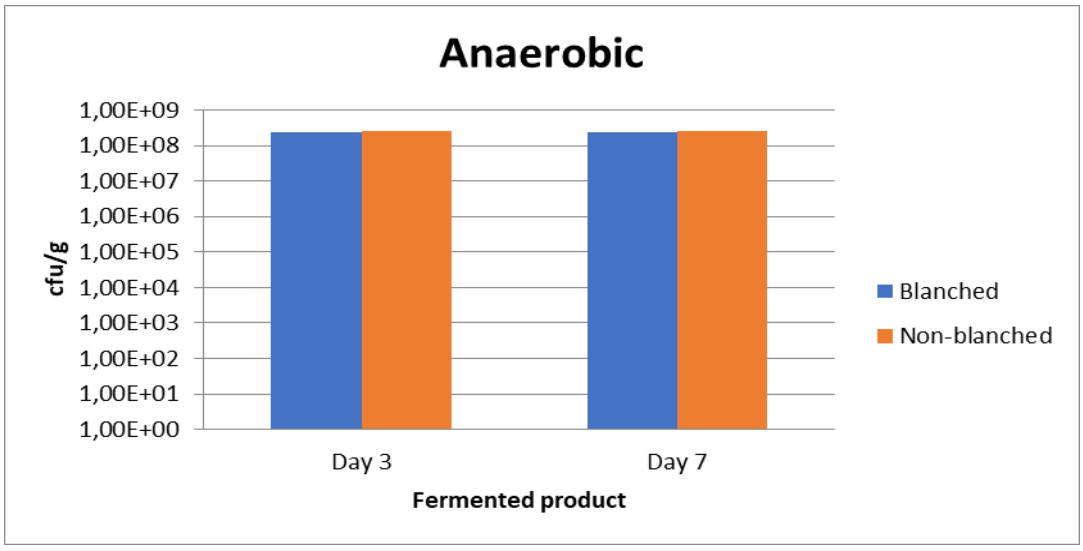

Figure 69: Anaerobic bacteria counts of the fermented paprika comparing a heat treatment vs. without

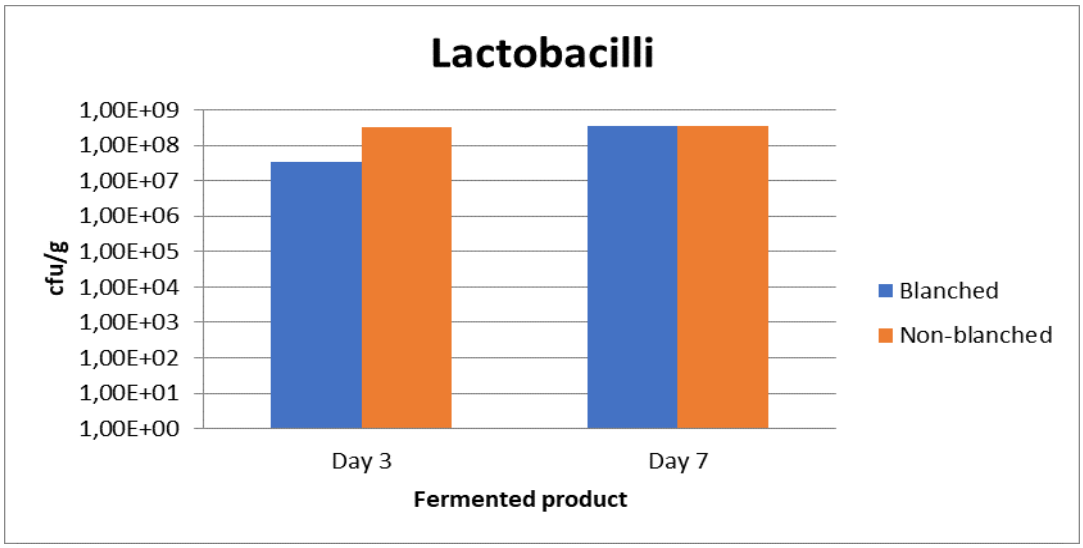

Figure 70: Lactic acid bacteria counts of the fermented paprika comparing a heat treatment vs. without

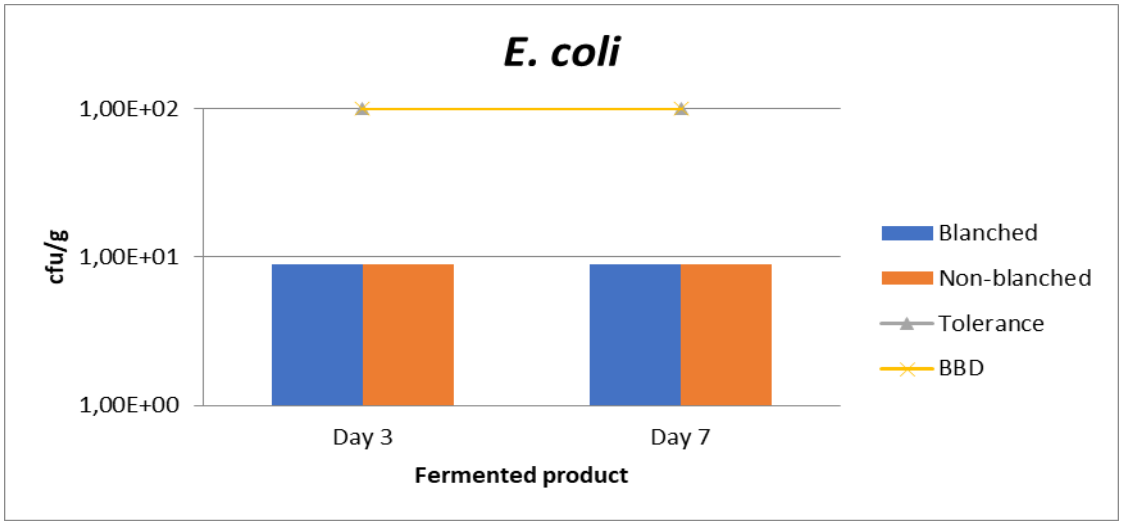

Figure 71: E. coli counts of the fermented paprika comparing a heat treatment vs. without

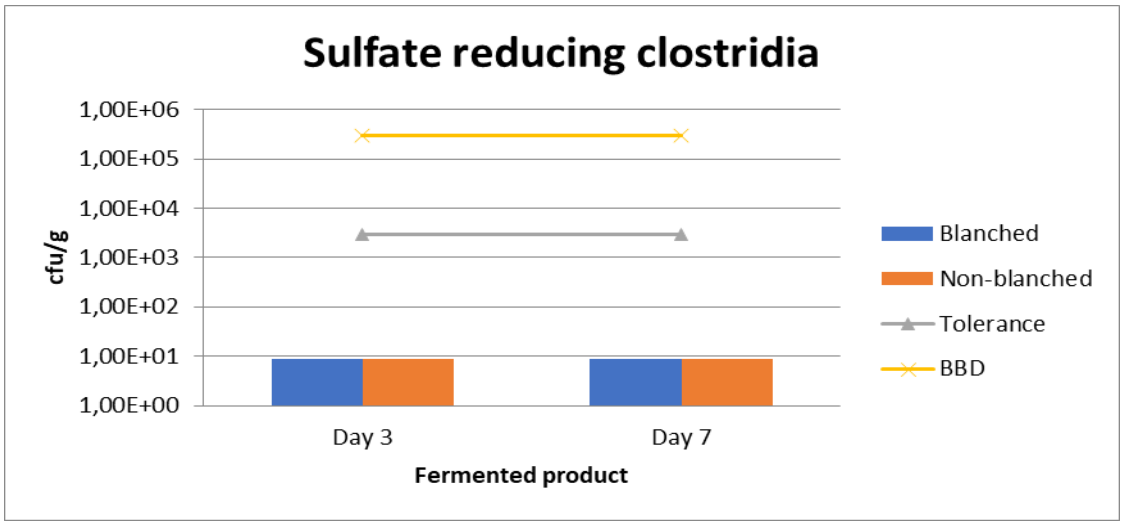

Figure 72: Sulphate reducing clostridia counts of the fermented paprika comparing a heat treatment vs. without

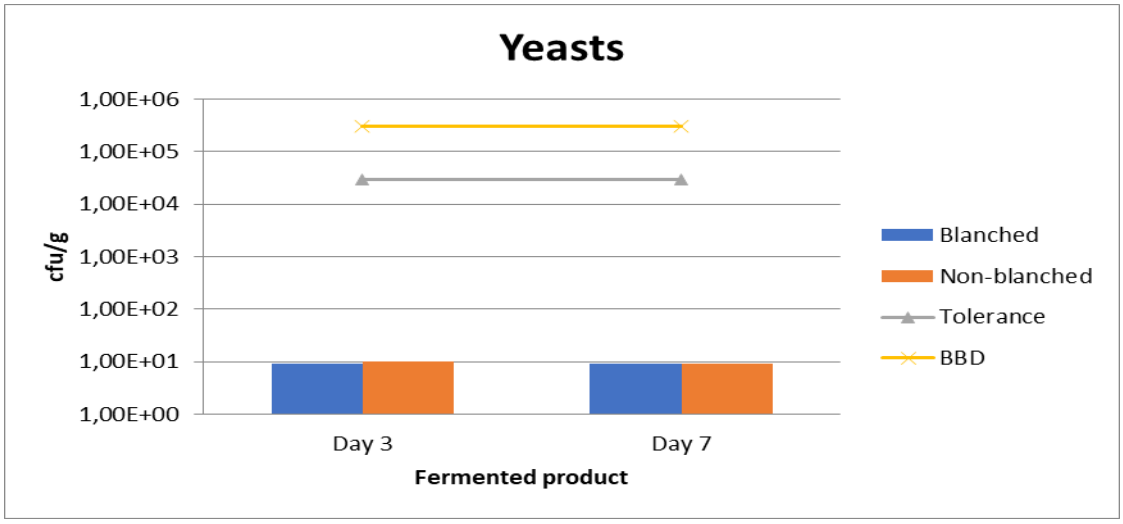

Figure 73: Yeasts counts of the fermented paprika comparing a heat treatment vs. without

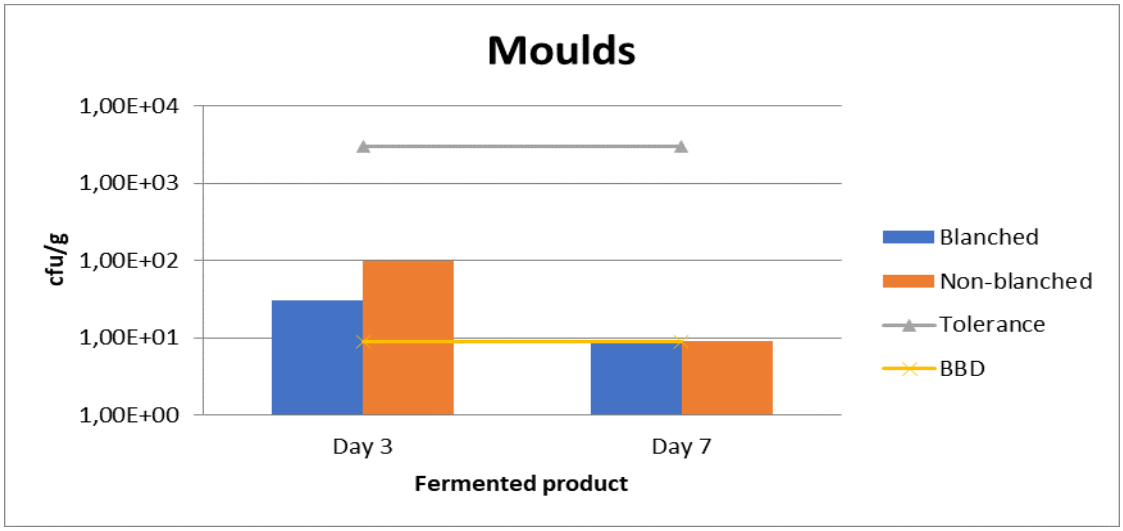

Figure 74: Moulds counts of the fermented paprika comparing a heat treatment vs. without

Discussion and conclusions

The results obtained in the experiments carried out remarks the importance of using fresh raw material and also the importance of a good fermentation process without cross contamination.

he washing method of the paprika was the same for all the experiments; wash and cut in cubes with a disinfected knife, but the raw material used wasn't; in the first experiment the paprika used was 2 days stored in the fridge. However, in the second experiment the paprika used was fresh and the same results have been obtained. The blanching treatment during 30 seconds improvement the stability of the product as the colour of the brine wasn't turbid, which probably will increase the shelf life too.

Spontaneous fermentation of this product doesn't occur always, which increases the importance of an inoculation to lead the fermentation process. In addition, the starter culture that mostly seems to work with this product is an inoculation with brine from previous fermentation of paprika as it gives the typical smell, aroma and taste of a fermented paprika.

In conclusion, the raw material needs to be free of any illness and as fresh as possible in order to achieve a successful fermentation process. Also the use of the same amount of brine and work always in same way and in same condition.

e. Spent grain

I. Induced vs. spontaneous fermentation – normal jars

Visual inspection:

In the visual inspection there weren't significant differences comparing the spontaneous with the induced fermentation. What was observed is that the spent grain absorbed all the brine added and consequently the spent grain weren't submerged in the brine (figure 75). The smell using a starter culture was acidic and in the spontaneous fermentation it was typically of a spoiled product. No taste test was carried out.

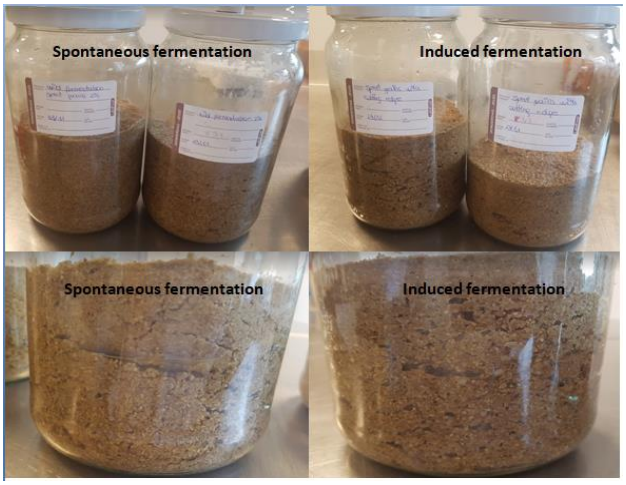

Figure 75: Fermented spent grains visual inspection on third day of fermentation

pH:

The pH of the fresh spent grain shows a clear tendency to increase, and so the importance to ferment them as soon as possible in order to prolong the shelf life The pH is found in a range where the bacteria can grow easily. The drop in the pH is faster using a starter culture rather than in the spontaneous fermentation. However, the spontaneous fermentation is not safe as the pH is above 4, 5. See figure 76 on the net page.

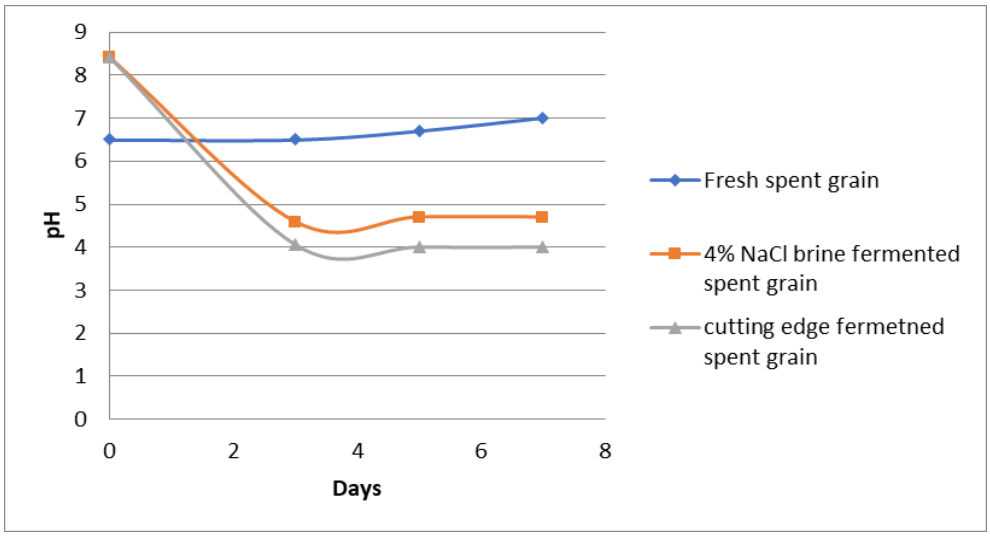

Figure 76: pH evolution of the fresh and fermented spent grain

Microbiology:

The microbiology results regarding the fresh spent grain shows the variety of its microbiota. On the first 5 days no visible mould growth has been detected despite the high amount of water that contains. However, other kind of bacteria have been isolated in the fresh spent grain by sequencing on the region 16S rRNA; based on 400 nucleotides (*Undibacterium* strains with a max score of 536, query cover of 91% and identity of 85%); based on 578 nucleotides (Enterobacteriaceae such as *Cronobacter turicensis* with a max score of 771, query cover of 90% and identity of 89%; Enterobacter spp stains with a max score of 965, query cover of 97% and identity 97%); based on 400 nucleotides(*Bacillus spp strains* with max score of 416, query cover of 99% and 92% of identity. *Salmonella*, *Listeria monocytogenes*, sulphite reducing clostridia and coagulase-positive staphylococci haven't been isolated. During the fermentation the same bacterial strains have been isolated despite the acidification that occurs during fermentation. See figures 77 to 81.

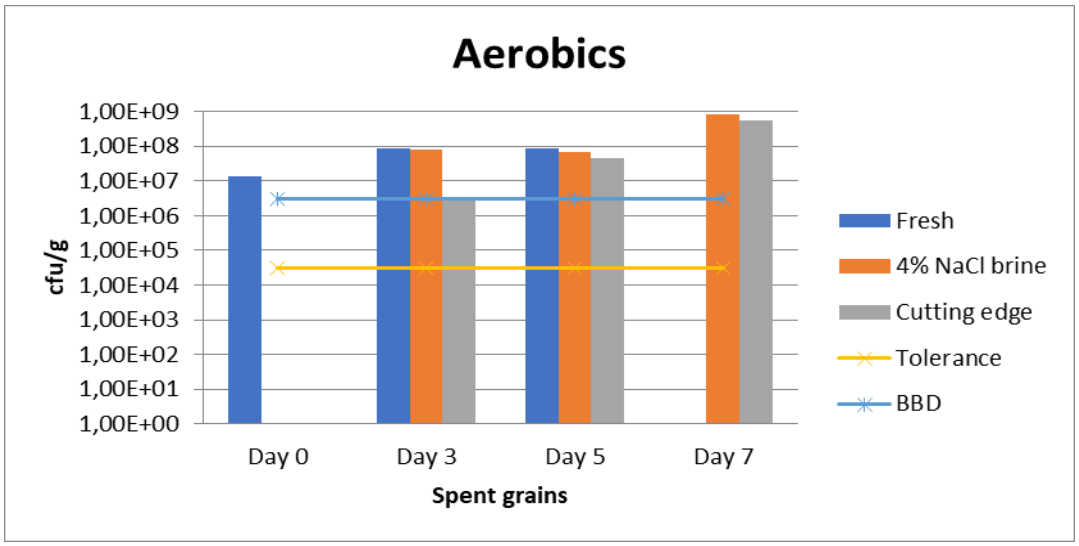

Figure 77: Aerobic bacteria evolution of fresh and fermented spent grains

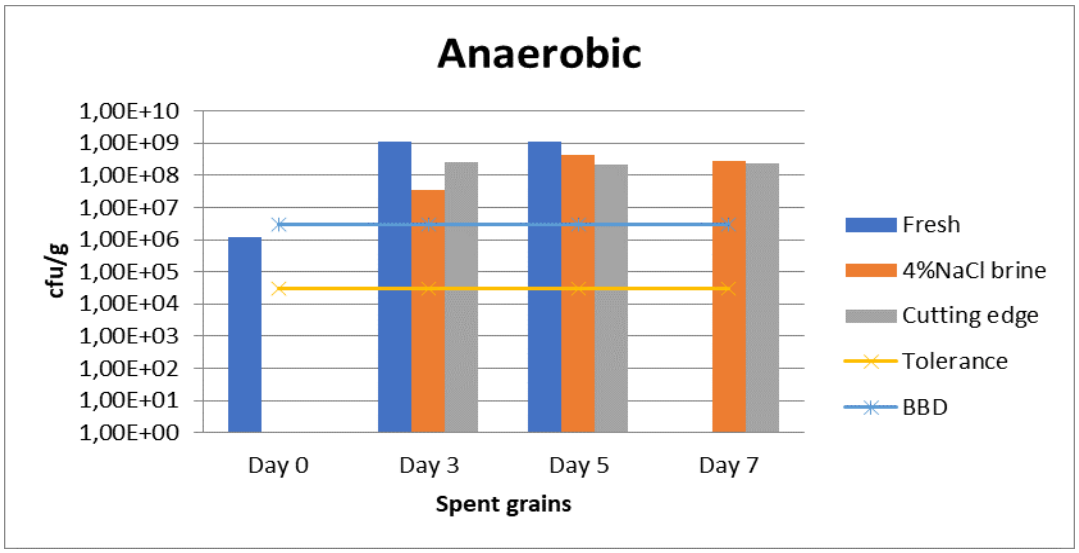

Figure 78: Anaerobic bacteria evolution of fresh and fermented spent grains

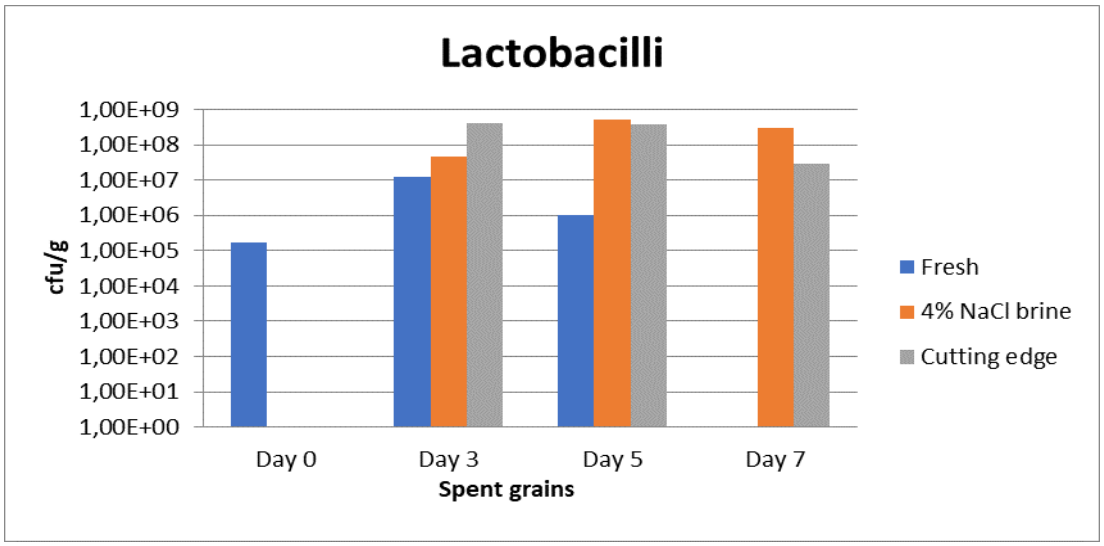

Figure 79: Lactic acid bacteria evolution of fresh and fermented spent grains

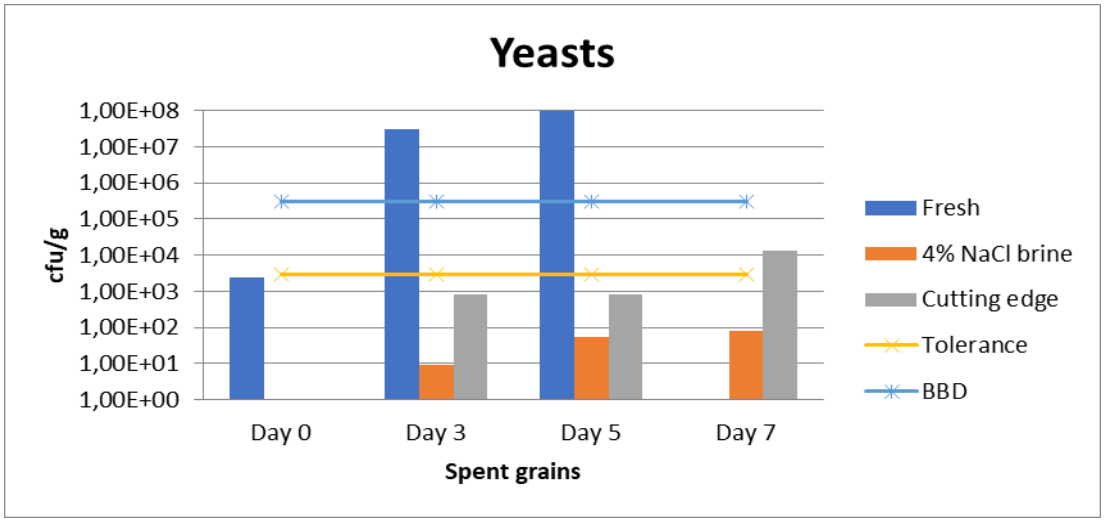

Figure 80: Yeasts evolution of fresh and fermented spent grains

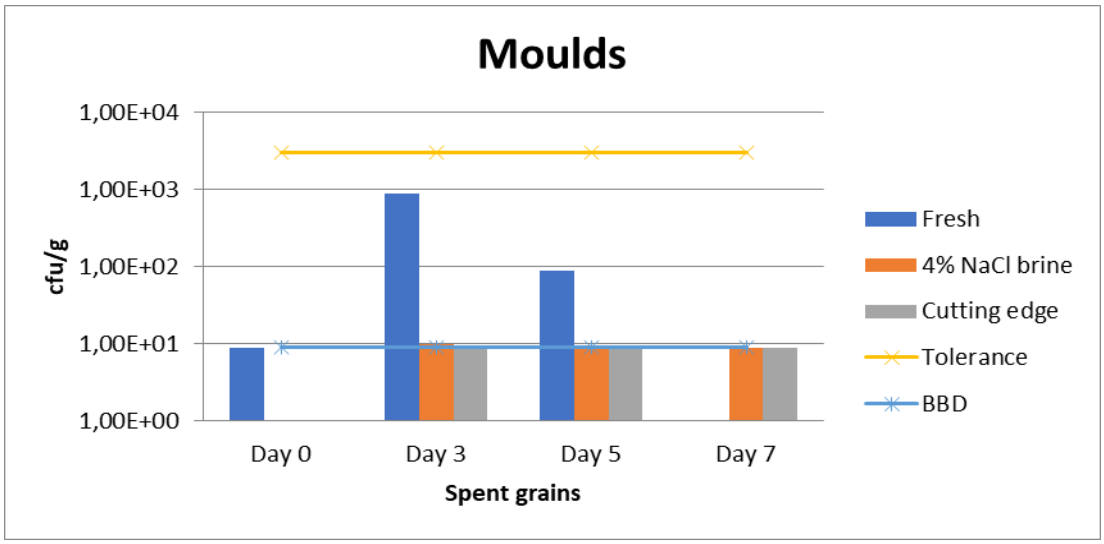

Figure 81: Moulds evolution of fresh and fermented spent grains (Note: The fresh spent grain were analysed only the day 0, 3 and 5

Discussion and conclusions:

However, nothing can be concluded yet as only one batch of fresh spent grains has been analysed and processed. A close study will be needed in order to obtain relevant information from different batches from the same and from different breweries.

Another batch of spent grain coming from another brewery has been tested for its shelf life. This batch of spent grain had a much higher moisture content than the spent grain batch used for fermentation. This shows that there is a very big difference between different types of spent grain. Therefore, it can be concluded that it will be necessary to test the specific type of spent grain to obtain an optimised fermentation process and to ensure the food safety.

3.5 Discussion and conclusions

Based on the current results, it can be concluded that induced fermentation always works, which is not the case for spontaneous fermentation. When spontaneous fermentation works, there are no fundamental differences between spontaneous and induced fermentation concerning the process flow and the end products obtained.

Certainly when older (less clean) raw material is used, a blanching step in combination with the use of a starter culture is recommended.

For fermentation, different starter cultures can be used, often with comparable results. Based on our tests, we obtained the best results with LAB as starter culture. Rye sourdough was the least good one regarding taste and visual aspects. The cheapest way to work with starter cultures is the back-slopping technique which means the reuse of a fraction of the previous production.

Fermentation is often used to extend the shelf life. Nevertheless not all fermentation processes lead to a significant shelf life extension. The fermented peppers had a shelf life of more than 6 months but with spent grains the shelf life stayed limited to only 6 days even after fermentation.

4. Drying

4.1 Introduction

Drying of foods is also well known as a stabilisation technology. Also when extraction of bioactive compounds e.g. cannot be performed directly on fresh fruits and vegetables, drying needs to be conducted to keep the materials for later use. Dried fruits and vegetables have also been regarded as alternative fat-free snacks and received more attention from the food industry during the past decade. This implies that not only nutritional changes, but also other changes including physical and microstructural changes are of importance and need to be monitored when applying drying processes (Devahastin & Niammy, 2010).

4.2 Aim

The aim of the stabilisation test was to evaluate the impact of the different drying technologies on the quality aspects of the dried herb stems. These herb stems are a real by-product of the vegetable & herb processing industry which remains underutilised up till now. The figure below shows the herb species studied.

Figure 82: Samples of the 4 herb species of which the stems where used for drying tests

4.3 Materials and methods

For the drying experiments, the leaves and stems of parsley (*Petroselinum crispum*), mint (*Mentha spicata*), coriander (*Coriandrum sativum*) and basil (*Ocimum basilicum*) were obtained from an industrial processor of fresh herbs into frozen products. The herbs were stored at -18°C before being subjected to the selected drying procedures.

The following drying techniques were chose for comparison: air drying, freeze-drying, refractive window drying, microwave-drying and vacuum-microwave drying. The leaf-part of the herbs were exclusively air-dried, whilst the stem-part was subjected to all five drying techniques.

a. Air drying

The leaves and stems of four kinds of herbs were air-dried in a multifunctional chamber. The herbs are placed on a number of shelves that are secured in a transportable trolley. The installation handles the principle of fresh air supply, which means outside air gets drawn in which is heated in order to control inner temperature.

ILVO
Flanders Research Institute for Agriculture, Fisheries and Food

Figure 83: Air dryer used at ILVO

All samples were subjected to air-drying with a temperature of 70°C for 5 hours. The principle of fresh air was used, meaning there was no active air circulation. Outside air was used by the machine to control its temperature.

b. Freeze-drying

Another portion of the stems were freeze-dried. The machine that was used has a batch capacity of 5 shelves with an area of 0,7 m², and an ice condenser capacity of 8 kg/24h. The minimum temperature of the ice condenser is -85°C and the temperature range of the shelves is -50°C to 120°C. All parameters are adjustable.

ILVO
Flanders Research Institute for Agriculture, Fisheries and Food

Figure 84: Freeze dryer used at ILVO

Parameters used for freeze drying the herb stems were:

Process step	Shelf temperature (°C)	Duration (h)	Absolute pressure (mbar)
Loading	-50	2	1000
Sublimation			
• **Step 1**	-50	1/6	1000 -> 0.16
• **Step 2**	-50 -> +20	20	0.16
• **Step 3**	+20	20	0.16
Desorption			
• **Step 1**	+ 20	1/6	0.16 -> 0.025
• **Step 2**	+ 20 -> + 30	1 + 5/6	0.025
• **Step 3**	+ 30	3	0.025

Ice condenser temperature: -90°C

c. Dry-On-Water

A third part of the herb-stems was taken for drying with a Dry-On-Water installation. This installation handles the principle of refractive window drying, with the addition of a cooling step when necessary (depending on glass transition temperature).

For preparation, the stems were cut in a vacuum-cooking cutter with a cutting speed of 6000 rpm and a bowl rotation speed of 14 rpm.

A homogenous product is formed into a film on top of a conveyer belt. It then moves over a warm-water bath adjustable in temperature, where the water gets evaporated and drawn into a ventilation system. After passing the warm-water bath, the conveyer belt moves on top of a cold-water bath to cool the product below the glass transition temperature. This ensures a good separation of the product and the belt material, where after the product can be collected as flakes.


Figure 85: Vacuum-cooking cutter and Dry-On-Water used at ILVO

To dry the coriander stems on the dry-on-water installation, the following parameters were used:

- Table angle: 5°
- Slit of applicator: 2 mm
- Temperature cold water bath: 50°C
- Belt speed: 15 cm/min
- Temperature warm water bath: 95°C
- Ventilation: 6000 m^3/h (maximum)

d. Microwave drying

Another sample of the herb-stems was used in microwave drying. In microwave drying the heat is generated by directly transforming the electromagnetic energy into kinetic molecular energy. The heat is generated deep within the fabric to be dried thus creating a temperature gradient towards the surface. The temperatures inside are higher than outside, giving rise to a higher partial pressure that drives the evaporating liquid to the surface.

The microwave used is a batch microwave, with a maximum power of 3kW, equipped with a rotating drum. The pressure is controlled by a vacuum pump and set at 750mbar. Power, pressure, rotation angle and speed of the drum are adjustable.

Figure 86: Microwave drying installation at VIVES. The same system was used for the Vacuum-microwave drying experiments

About 500 g of frozen herb-stems were placed directly in the product drum and the drying process was started immediately. The drying parameters are displayed in the table below:

drum speed 2 rpm
drum angle 100°C
pressure 750 mbar

	parsley stems		**mint stems**		**coriander stems**		**basil stems**	
	time (min)	P set (kW)	time (min)	P set (kW)	time (min)	P set (kW)	time (min)	P set (kW)
drying process	0,5	0,5	0,5	1	0,5	0,5	0,5	1
	8	1,5	8	1,5	8	1,5	9	1,5
	18	1	18	1	19	1	18	1
	18	0,5	10	0,5	19	0,5	12	0,5
	20	0,3	8	0,3	19	0,3	13	0,3
							9	0,2
absorbed energy	0,67 kWh		0,58 kWh		0,67 kWh		0,63 kWh	
duration	64,5 min		44,5 min		65,5 min		61,5 min	
max temp	77°C		85°C		71°C		76°C	

e. Vacuum-microwave drying

A fifth portion of the herb-stems was dried using the vacuum-microwave drying technique. The same batch microwave was used as for microwave drying but the pressure was set at 10 mbar during the whole process resulting in a lower drying temperature.

About 500g of frozen herb-stems were placed directly in the product drum and the drum was placed in the vacuum chamber. When a vacuum of 10 mbar was reached the following drying procedures were conducted:

drum speed 2 rpm
drum angle 100 °C
Pressure 10 mbar

	parsley stems		**mint stems**		**coriander stems**		**basil stems**	
	time (min)	P set (kW)	time (min)	P set (kW)	time (min)	P set (kW)	time (min)	P set (kW)
drying process	21	1	22	1	23	1	23	1
	5,5	0,7	8	0,7	5	0,7	5	0,7
	14	0,5	4,5	0,5	7,5	0,5	7,75	0,5
	5	0,3	12	0,3	12	0,3	12	0,3
absorbed energy	0,5 kWh		0,5 kWh		0,5 kWh		0,5 kWh	
duration	45,5		46,5 min		47,5 min		47,75 min	
max temp	62°C		60°C		60°C		62°C	

f. Analyses

To investigate and compare the effect of the drying techniques, samples were analysed for moisture, water activity, colour, polyphenolic content and aroma profile.

Moisture, water activity and colour

Moisture content of dried samples was measured using a HC103 Halogen Moisture Analyzer (Mettler Toledo, Belgium).

For water activity, a bench-top water activity meter was used (AQUALAB 4TE). Samples were analysed in triplicate.

Additionally, powdered samples were taken for colour measurements using a Bench-top Colorimeter CR-5 (Konica Minolta). The petri-dish/reflectance method was used. Using the CIELAB colour space, values for L*, a* and b* were noted and colour differences (ΔE) were calculated using the formula:

$$\Delta E = \sqrt{(\Delta L)^2} + (\Delta a)^2 + (\Delta b)^2 \quad (1)$$

Relative evaluation of the colour difference was made in accordance with the following statements[2]:

ΔE ≤ 1: Not perceptible by the human eye
ΔE = 1-2: Perceptible through close observation
ΔE = 2-10: Perceptible at a glance
ΔE = 11-49: Colours are more similar than opposite
ΔE = 50-99: Colours are more opposite than similar
ΔE > 100: Colours are exact opposite

Polyphenolic content

Fresh herbs are an excellent source of polyphenolic compounds, that have shown to possess health-promoting traits. To investigate the influence of different drying techniques, a LC-MS analysis was used to search for certain polyphenolic compounds and quantify them.

To prepare the samples for analysis, an extraction protocol using methanol (MeOH) was used. For this, 0,5 ± 0,001 g of the sample was weighed in a test tube, wetted with MeOH and combined with the internal standard diazine. After extraction including sonication and centrifugation (2000 x *g*, 5 min), supernatans was filtered over a PVDF syringe filter for testing. Analysis was done on a Acquity UPLC® - Xevo TQ-XS (LC-MS/MS) in MRM mode. Data processing was done with the use of the program Targetlynx.

ORAC Antioxidant Capacity Assay

In the ORAC assay, the radical initiator component 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) decomposes spontaneously at 37 °C to form 2 carbon-centered radicals which react with oxygen to generate peroxyl radical, a common radical in human biology. Fluorescent probes used in this assay decompose in a pattern that is consistent with the HAT mechanism of action when exposed to peroxyl radicals (Prior et al., 2005). The assay was carried out as described by Bernaert et al. 2012.

Aroma profile

Lastly, aroma components were analysed in dried samples using GC-MS. For this, samples were weighed (0,5 ± 0,1 g) in SPME vials and capped. The vials are loaded in the machine as such.

The headspace (gas phase present above the sample) was sampled during 30 minutes with a DVB/CAR/PDMS fibre at room temperature. This was subsequently analysed with GC-MS. The program used was SPME-Kamertemp-30min-normal 1D mode. The column was a DB-5MS + RG (35 m x 250 µm x 1 µm)

On one hand, data processing was done with the use of Unknown analysis, which handles the principle of identifying aroma compounds based on MS spectrum and retention index, compared to in-house information or that from commercial libraries.

[2] Source: http://zschuessler.github.io/DeltaE/learn/

On the other hand, processing of results was done with MS Quantitative analysis (to check integration), using Agilent.

4.4 Results

Moisture, water activity and colour

Table 5 shows the data of the moisture content analysis of the 4 fresh and dried herb species (mint, parsley, coriander and basil). The moisture content in the fresh herb leaves varies between 82-89%, while in the stems this is less variable and always around 90 %. All drying methods resulted in a significant decrease of the moisture content, as expected. For most species the maximum moisture content level in the dried products of 10% was reached, only in few cases the remaining moisture content after drying was higher (e.g. microwave drying of mint stems). Moisture content in the stems dried with microwaves or vacuum-microwave was always in the higher range for three out of the four species tested: parsley, mint and coriander.

Sample	Moisture content (%)
Munt blad vers	88,71
Munt - Luchtgedroogd	6,11
Peterselie blad vers	82,61
Peterselie - Luchtgedroogd	2,56
Koriander blad vers	83,00
Koriander - Luchtgedroogd	2,68
Basilicum blad vers	86,77
Basilicum - Luchtgedroogd	6,24

Sample	Moisture content (%)
Muntstelen vers	90,06
Muntstelen - Luchtgedroogd	4,00
Muntstelen - Gevriesdroogd	9,04
Muntstelen- Microgolf	13,02
Muntstelen - Vacuüm microgolf	7,87
Peterseliestelen vers	90,64
Peterseliestelen - Luchtgedroogd	3,85
Peterseliestelen - Gevriesdroogd	3,64
Peterseliestelen - Microgolf	9,71
Peterseliestelen - Vacuüm microgolf	8,50
Koriandersteln vers	90,15
Koriandersteln - Luchtgedroogd	3,03
Koriandersteln - Gevriesdroogd	4,14
Koriandersteln - DOW	2,93
Koriandersteln - Microgolf	8,87
Koriandersteln - Vacuüm microgolf	12,11
Basilicumstelen vers	89,48
Basilicumstelen - Luchtgedroogd	6,19
Basilicumstelen - Gevriesdroogd	5,01
Basilicumstelen - Microgolf	6,23
Basilicumstelen - Vacuüm microgolf	6,41

Table 5: Moisture content of fresh and dried herbs, both for the leaves (commercial product) and the stems (by-product)

It is clear from Table that all dry products have a a_W way below 0.6, which is the absolute limit value of microbial growth. The dried products thus can be considered as shelf-stable products if stored appropriately.

In Table 6 also the results of the colour analysis is given, together with the a_w values of all the dried products.

COLOUR VALUES

SAMPLE	L*	a*	b*	a_w	Moisture (%)
M-L	30,37	0,17	12,56	0,2837	6,11
MS-L	48,73	3,01	20,12	0,2480	4,00
MS-V	61,87	-0,96	22,25	0,5003	9,04
MS-MG	45,15	3,01	20,58	0,5509	13,02
MS-VMG	54,14	-0,77	22,10	0,2603	7,87
P-L	39,20	-3,27	18,31	0,2225	2,56
PS-L	52,68	-4,67	23,01	0,2303	3,85
PS-V	64,99	-8,47	24,85	0,1769	3,64
PS-MG	51,24	-1,49	22,58	0,3052	9,71
PS-VMG	54,34	-8,30	24,81	0,2890	8,50
K-L	30,93	-1,15	14,08	0,2067	2,68
KS-L	40,52	-1,10	18,23	0,2286	3,03
KS-V	56,46	-8,14	26,44	0,1845	4,14
KS-DOW	39,00	-1,74	22,33	0,2139	2,93
KS-MG	40,86	0,42	18,97	0,3005	8,87
KS-VMG	44,18	-4,79	21,10	0,3104	12,11
B-L	37,34	-0,87	20,13	0,2910	6,24
BS-L	37,34	-0,87	20,12	0,2814	6,19
BS-V	59,33	-8,07	26,91	0,2049	5,01
BS-MG	43,38	0,01	18,04	0,2897	6,23
BS-VMG	47,93	-5,57	21,08	0,1799	6,41

Table 6: Results for colour values (L,a*,b*), water activity (a_w) and moisture content of dried herb leaves and stems. Nomenclature: M = Mint; MS = Mint stems; P = Parsley; PS = Parsley stems; K = Coriander; KS = Coriander stems; B = Basil; BS = Basil stems. L = Air dried; V = Freeze-dried; DOW = Dry-On-Water; MG = Microwave-dried; VMG = Vacuum-Microwave-Dried*

Talking about colour, it was already obvious from the visual inspection that there where clear colour differences both between the fresh leaves and stems (Figure 14). This is still easily detectable, also after drying as illustrated in Figure 8 for the dried parsley stems.

Figure 14: Visual appearance on fresh herb leaves and stem samples

Figure 88: Visual appearance of the dried parsley stem samples. Left: air dried; Middle: freeze dried; Right: microwave dried. Upper panels: sample without milling; Lower: samples after milling.

The graphs in Figure 9 illustrate in more detail the colour data collected on the dried samples and allow comparison of the impact of the different drying techniques. For Coriander only, the DOW technology could be evaluated. This is due to the fact that this innovative drying technology is dependent on the fact that the product to be dried has to have the possibility to be spread in a thin layer in order to undergo an efficient Dry-On-Water process.

Without the use of enzymatic treatments of additional mixing, milling or homogenization process steps this wat not possible for the mint, parsley and basil stems. As these unit processes would have a huge impact on the process cost, they were not included in the tests.

Figure 89: Comparison of the colour analysis of the herb stems of the 4 species dried with the different drying technologies. Upper left: parsley stems; Upper right: mint stems; Lower left: basil; Lower right: Coriander.

Polyphenolic content

Besides analysis of the most relevant macroscopic parameters, some preliminary analysis were performed to have an idea about the impact of the different drying technologies on some bioactive compounds. Herbs are known as aromatic plants, rich in bio-actives such as polyphenols. In addition the presence of polyphenols also contributes to the potential as antioxidant in food preparations. A validated LC-MS method running at ILVO was used to estimate the amount of 40 different phenolic acids and polyphenols in the dried samples. As an example, Figure , shows the results of this analysis for 5 different phenolic compounds analysed in the dried coriander stems. The generated dataset allows us to evaluate, in great detail, the impact of the used drying technologies on the phenolic compounds in the herb stems. The entire data set is available upon request.

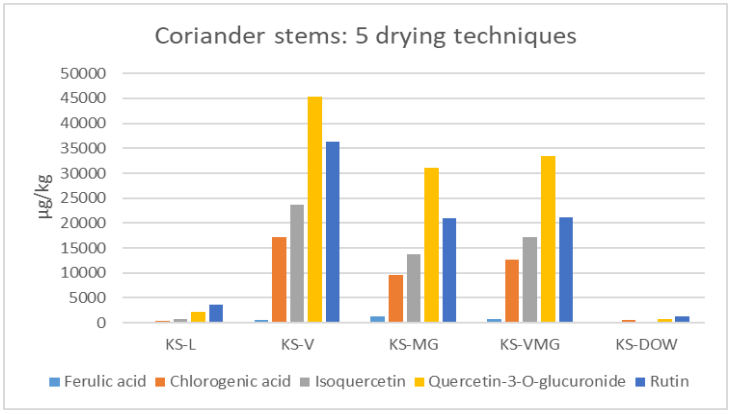

Figure 90: Content of five different phenolic acids and polyphenols in dried coriander stems. L = air drying; V = freeze drying; MG = microwave drying; VMG : vacuum-microwave drying;

ORAC Antioxidant capacity assay

The ORAC assay was used to investigate the antioxidant potential of the dried herb stems. Herbs are known as aromatic plants, rich in antioxidants. In general, comparing all ORAC values obtained, the dried mint stems showed the highest ORAC values compared to the other herb species (coriander, parsley and basil). This is also illustrated by the fact that the ORAC values of the microwave dried and freeze dried samples of the mint samples, 80 x fold diluted, where still too high and fell out of the quantification range. Further dilutions need to be tested in order to assess these ORAC values in a reliable way. In Figure the ORAC values of the different herb stems all dried with hot air (70°C for about 5h) were compared. When only looking at hot air dried samples, the parsley samples showed the highest ORAC antioxidant potential.

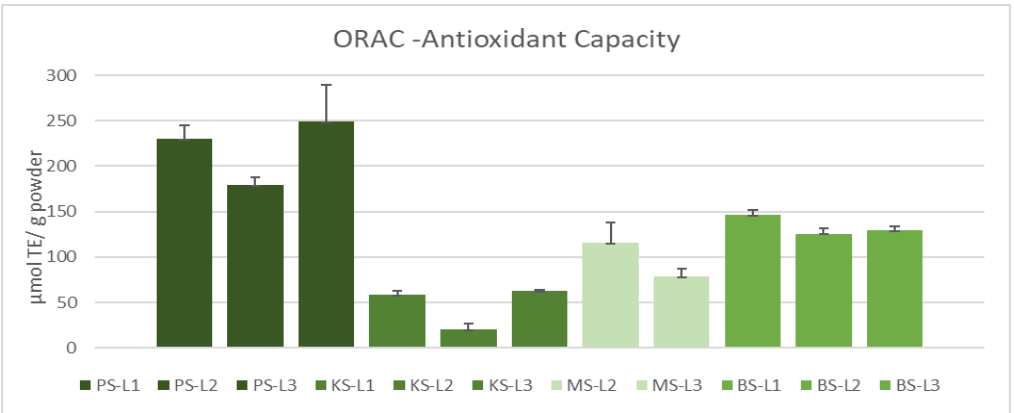

Figure 91: ORAC values of the air-dried stem samples of the four herb species parsley (PS), coriander (KS), mint (MS) and basil (BS). All samples were analysed in triplicate. The signal of one mint sample (MS-L1) was too high and fell out of the detection range. Additional dilutions of this sample need to be analysed.

In Figure the ORAC values of the coriander stems, dried with five different technologies are compared. From these results it is evident that both the microwave assisted drying (± 129 µmol TE/ g powder) as well as the microwave-vacuum assisted drying (± 153 µmol TE/ g powder) perform at least as good as freeze drying (± 121 µmol TE/ g powder). Air drying (± 47 µmol TE/ g powder)and Dry-On-Water drying (± 56 µmol TE/ g powder) resulted in much lower ORAC values. However, this is a conclusion that cannot be generalized. When the same exercise is done for the parsley stems e.g. on average the ORAC values of the air dried samples are in the same range (± 219 µmol TE/ g powder) as the parsley stems dried with the microwave-assisted drying ((± 213 µmol TE/ g powder) and microwave-vacuum assisted drying method (± 197 µmol TE/ g powder).

In conclusion, it is thus hard to predict which drying technology is best in order to retain the antioxidant capacity in the dried product as it seems to be dependent on the interaction between herb-species and technology used.

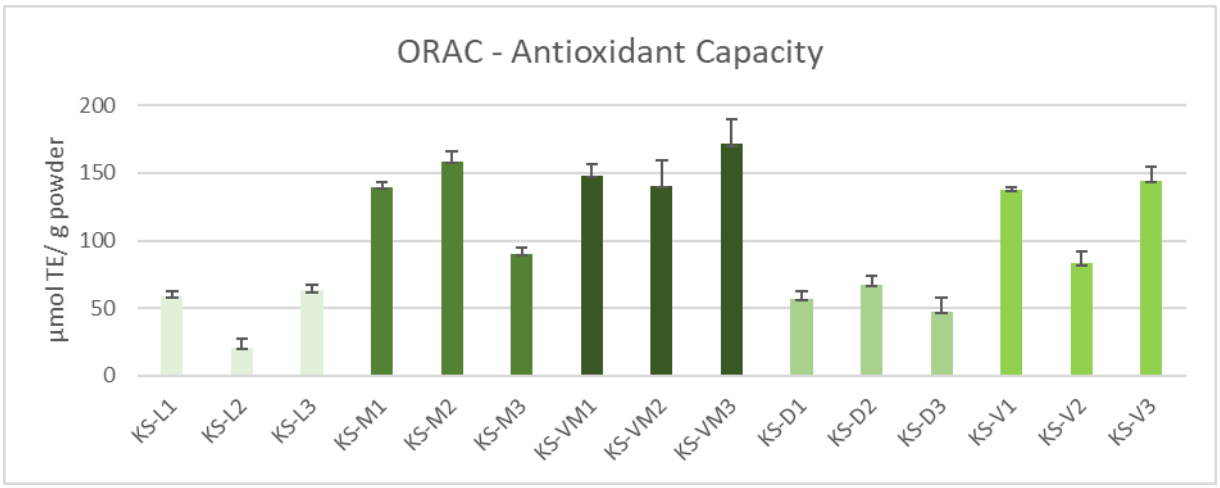

Figure 92: ORAC values of the coriander stems, dried with five different technologies, i.e. hot air drying (L), microwave assisted drying (M), microwave-vacuum assisted drying (MV), Dry-On-Water (D) and freeze drying as reference technology (V).

Aroma profile

Finally, the aroma of herbs and spices is another relevant parameter connected to product quality. For that reason also a GC-MS analysis of the volatile aroma compounds was carried out. This aroma profile was determined for both the fresh and the dried herb samples. This dataset allows us to estimate the potential contribution of the dried herb stems when used as a technical (colourant e.g.) or functional ingredient (antioxidant e.g.).

As an example the results of the GC-MS analysis for three typical mint aroma compounds is shown figure 93), both for the air dried mint leaves and stems. D-limonene is contributing to the perception as a citrus, orange fresh sweet note. Carvone is described as a minty licorice, while humulene has the typical woody note as description.

For these three compounds the GC-MS analysis revealed that they are much less abundant in the dried stems, compared to the dried leaves.

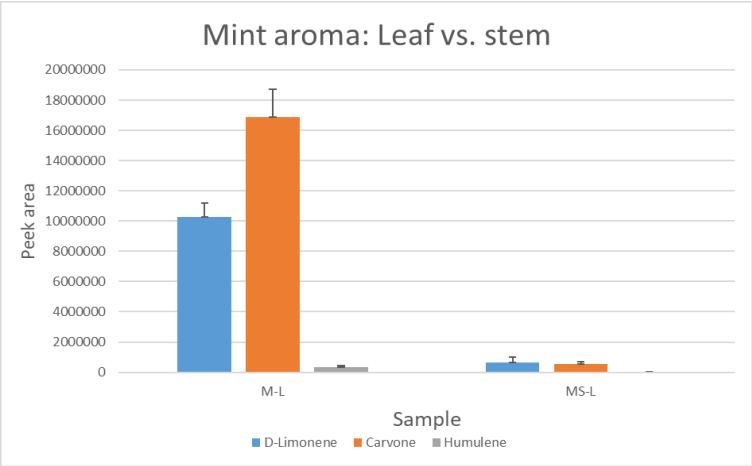

Figure 93: GC-MS results of three important mint aroma compounds, analysed both in air dried leaves and stems.

4.5 Discussion and conclusions

Based on the current results of the herb stem case study it is evident that the quality of the dried end product is hard to predict. All drying technologies resulted in a sufficient decrease in moisture content and a sufficiently low a_W value, characteristic for shelf-stable products.

Freeze drying and vacuum-microwave drying led to a higher L*-value, hence brighter, more appealing products in most cases, except for parsley. Freeze drying and vacuum-microwave drying also led to a lower a*-value in the basil and coriander samples, being greener. On the other hand air drying and microwave drying led to a lower b*-value, which can be interpreted as more blue, i.e. contributing to a darker colour.

From the analysis of the anti-oxidant capacity of the dried herb stems via the ORAC test, it is evident it is hard to predict which drying technology is best in order to retain the antioxidant capacity in the dried product as it seems to be dependent on the interaction between herb-species and technology used.

Finally, both the LC-MS and GC-MS data sets generated will be very valuable for the future activities in WP3 focussed on process and product development making use of the stabilized horticultural by-products.

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