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BioBoost
Accelerating biobased horticulture

Pretreatment methods and techniques

(WP3)

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ILVO
Flanders Research Institute for Agriculture, Fisheries and Food

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BioBoost - WP3 Report - Pretreatment methods and techniques (updated march 2020)

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Summary

The ever-growing demand for fresh food and an elevated awareness by the general public in healthy foods has significantly increased the production and processing of agricultural crops during the last decades. Along with it came a rise in the generation of large amounts of undesired by-products and food waste fractions.

For the BioBoost task on pretreatment methods and techniques (wp3), ILVO Flanders Institute for Agriculture, Fisheries and Food Research and VIVES University of Applied Sciences explored the valorisation of discarded or underused by-products and food waste fractions as well from the field as from the food industry and retail. New applications were developed for a series of selected by-products to explore pre-processing methods and create semi-finished and fully processed foods with a high potential for market uptake.

What these by-products are and how they can still be valorised is highly dependent on the type of crop and its production methods. Because of this, a case by case approach was necessary to identify and characterise the generated by-products and develop specific processes and applications for them. Qualitative by-products and food waste fractions that are underused or treated as waste can still be processed to obtain sustainable ingredients with applications in a wide variety of applications as well as non-food.

By-products from a selection of vegetables (Cabbage, White cabbage, Brussels sprouts, Broccoli stems, Kale, Belgian Endive roots, Chicory roots, Oyster mushroom stems, Black salsify, Pointed Sweet Bell Pepper) were characterised to extract fibres and determine their content in bioactive compounds. Additionally, the press cakes from the apple juicing industry, residual cooking fluids from the chickpea and soy-industry, and rejected class II pointed sweet bell peppers were characterised and the pre-processing potential of edible brassica varieties to obtain a minimally processed stable and tasteful purée was largely investigated.

ILVO and VIVES also studied and developed novel value-added applications from by-products of the fruit and vegetable industry by using them in specially adapted recipes. New recipes for tomato juices using class II tomatoes were created and tested by ILVO Food Pilot jointly with Tomabel, a Belgian quality label for tomatoes. These juices were critically assessed by a taste panel and the stability was monitored over the course of one year. Novel recipes were developed with the residual cooking fluids from industrial processing of legumes as an egg replacement to make chocolate mousses and vegan cheese, jams and jellies were made with class II pointed sweet bell peppers, a sweet bell pepper ketchup and chutney was developed, yacon root pulp ice cream was made by Vives as well as cookies from spent grain from the brewing industry ... and many more.

Finally, a microbiological assessment under storage conditions has been done by ILVO for some of the novel by-product-based creations to gain insights on their shelf-life.

General introduction

Fruits and vegetables are consumed raw, as minimally processed preparations or as fully processed foods and are characterised by their richness in various nutrients and bioactive compounds. However, the cultivation and production processes that are needed to obtain first grade fresh market products also generate high amounts of by-products and food-waste fractions. These by-product fractions are highly dependent on the kind of crop that is cultivated and the specific processing steps that the raw material is subjected to. Therefore, a case by case approach is needed to identify and characterise the by-product flows and develop specific processes to valorise them in food grade applications.

Many of these by-products are of high quality and remain food-grade as long as basic hygiene and storage and/or handling procedures are followed. Two large categories of by-products can be identified, on one hand the by-products and food-waste fractions that are produced during the cultivation and harvesting process (e.g.: the removal of unused parts such as leaves, stems, roots etc..), and on the second hand the by-products that are composed of harvested crops or parts of harvested crops that are discarded as a result of strict selection and quality assessment criteria. Crops with a deviating shape, colour or size in comparison to the conventional standard may not be suited for sale despite the intrinsic nutritional value of the product. These discards are usually labelled as a certain class, first class (class I) being the best and most visually appealing items, second class (class II) being the divergent ones (still often sold as cheaper alternatives in discount stores and used for processing, but all too often simply discarded as waste). However, second class fruits and vegetables are still qualitatively as good as the first-class products, and could be used to create processed or semi-finished products in which the original source material is no longer recognisable.

But also, later on in the cycle, by-products and waste fractions may be produced, for example as a result of a preparation process to obtain a finished or semi-finished product in the food industry. One example of this is the cooking water from chickpeas that can be used to replace eggs in certain vegan products as vegan chocolate mousse. Another one studied is the press cake that is generated from the pressing of fruits to obtain a fresh juice, leaving the remaining flow unused despite it is still containing dietary fibres, enzymes, vitamins, minerals, oils and other interesting bio-active compounds such as carotenoids, polyphenols and others. These compounds can be extracted and purified from the original residual flow and utilized in different industries including the food industry, for the development of functional or enriched foods, the health industry for medicines and pharmaceuticals, as an ingredient in cosmetics, the textile industry, etc... The utilisation of by-products and food waste flows that would otherwise be regarded as waste for the creation of new value-added products fits within the ever-growing awareness towards sustainable development while restricting the amounts of waste that requires to be processed or disposed of. It also opens up new economic

opportunities to develop new products and to create new cost effective and value-added applications.

This report describes and explores the valorisation opportunities and methods of different kinds of agricultural and horticultural crop by-products and food waste fractions within the Belgian context and seeks to develop and provide new applications for a selection of these by-products and food waste fractions that are currently insufficiently utilised despite their great potential for new applications in both the food industry and other non-food markets.

1. Pre-processing

1.1. Introduction

Pre-processing is a manipulation of the raw product to make it suitable for further processing. In our case these raw materials are carefully selected by-products or food-waste fractions that undergo a first processing step to make them suitable for further use as a primary ingredient for the preparation of food, feed or other sustainable food-related applications, rather than to discard them. An important aspect of pre-processing is to characterise the used by-products. This is relevant since processing of by-products is relatively new and very little is still known on their nutritional composition, presence of bioactive compounds or food safety. It is important to only use food-safe products that don't contain harmful components. One example is the Belgian endive, from which only the forced leaf bud is used as a vegetable for cooking. The roots are discarded but may still be valuable to be used for other food applications. Since the composition of the by-products may deviate from that of the marketed produce, it is important to know in advance what the composition of the by-products is and to evaluate their safety for potential use in the food chain.

Pre-processing of by-products may also refer to the extraction of an interesting component or a specific fraction from the initial source material. Examples are the extraction of fibres from roots, stems, or leaves, or the purification of interesting components.

1.2. Aim

The aim of the pre-processing part is to explore the processing potential of by-products that can be utilised for human food applications. A selection of by-products was characterised to evaluate their potential as a source of fibres and other components for further processing. On the other hand, various pre-processing techniques for minimally processed by-products such as drying, various time/temperature heating combinations or techniques for separating targeted fractions from the initial by-product stream were evaluated.

1.3. Case studies

1.3.1. Fibre extractions from a selection of 10 vegetables by-products and food waste fractions

Developed at ILVO

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ILVO
Flanders research institute for agriculture, fisheries and food

Introduction

By-products from ten selected vegetables, (Cabbage, White cabbage, Brussels sprouts, Broccoli stems, Kale, Belgian Endive roots, Chicory roots, Oyster mushroom stems, Black salsify, Pointed Sweet Bell Pepper) were chosen based firstly on the degree of available by-product production for each vegetable species, and secondly on information from the literature on the presence of fibres with probable some bio-activity, e.g. pectin, inulin, lignin, cellulose and hemicellulose, resistant starch, fructo- and galacto-oligosaccharides. Furthermore, the proven presence of bioactive substances with a potential health benefit were also taken into account, including components with an antioxidant activity, polyphenols, glucosinolates, etc.

The selected by-products were subjected to a physical pretreatment and further processed into a final product, namely a soluble and insoluble fibre fraction. These fibre fractions were produced with the aim of further investigation into the health effects that they can bring forth in the microbiome of the consumer, such as bio-active function (of the soluble fraction) and prevention of constipation by faecal bulking (insoluble fraction).

After the production phase, the fractions were thoroughly investigated with the focus on ingredients that are linked to further potential health benefits for the consumer, e.g. polyphenols, resistant starch etc. Finally, an innovative application was sought for meaningful use of the fibres in food.

The main objective of this research is the valorisation of vegetable by-products and the reduction of unnecessary food waste, with the additional focus being a contribution to consumer health.

Methods

By-products from vegetables

The vegetable by-products used for this process are:

- ✓ Kale (*Brassica oleracea* convar. *acephala* var. laciniata)
- ✓ Savoy cabbage (*Brassica oleracea* var. sabauda)

- ✓ White cabbage (*Brassica oleracea* convar. capitata var. alba)
- ✓ Brussels sprouts (*Brassica oleracea* convar. oleracea var. gemmifera)
- ✓ Broccoli stems (*Brassica oleracea* convar. botrytis var. italic)
- ✓ Endive roots (*Cichorium intybus* var. foliosum)
- ✓ Chicory roots (*Cichorium intybus* var. sativum)
- ✓ Oyster mushroom stems (*Pleurotus ostreatus*)
- ✓ Black salsify (*Scorzonera hispanica*)
- ✓ Pointed sweet bell pepper (*Capsicum annuum*).

Pretreatment

Nearly all vegetables were acquired fresh, with the exception of kale (freshly frozen) and black salsify (blanched and cooled). They were subjected to an appropriate pretreatment, including a rinsing and comminution step, before air-drying at 60°C in a multifunctional cabinet. After the drying process, the vegetables were ground with an Ultracentrifugal Mill (Retsch), with a screen size of 750 µm. This size was found to be the lowest possible and available sieve size capable of grinding all residual streams (certain residual streams could not be successfully ground with a screen size of 250 µm). After grinding, the powders were divided into vacuum bags and aluminium bags, and stored in a dark room at 20°C until the purification and necessary analyses were performed.

Fractionation and purification

The next step was a water-based fractionation of the powders in an insoluble and soluble fraction, based on the method of Dalgetty and Baik (2003). This was done by (2x) repeated centrifugation (15 min 5000 x g) with demineralised water. The supernatant and the pellet were collected separately for the next purification step.

Soluble fraction

The supernatant was subjected to protein precipitation by means of pH adjustment with NaCl and HCl. First, the pH was brought to 3 using HCl. The liquid was left on a magnetic stirrer for 15 minutes to allow the reaction to take place. The pH was then adjusted to 9, so that a precipitate could be formed. After an additional 15 minutes, the liquid was centrifuged 2-3 times (20 min, 5000 x g), depending on the visible amount of precipitation. If necessary, and to remove all insoluble particles with certainty, a vacuum filtration was applied (Whatmann 40 filter paper).

The necessary samples were analysed for protein content for control. The soluble fraction was stored as such at 4°C until the next membrane filtration step.

Membrane filtration

To remove the mono- and disaccharides from the soluble fraction, membrane filtration, specifically nanofiltration, was used. Nanofiltration is a membrane filtration-based method that uses nanometer sized pores passing through the membrane. The membranes typically have pore sizes ranging between 1-10 nanometers. These are smaller than the ones used in microfiltration and ultrafiltration, but just larger than what is used in reverse osmosis.

The Molecular Weight Cut Off (MWCO) of the membrane was 300 Dalton (Da). Diafiltration was performed during each filtration, followed by a concentration. Diafiltration is a dilution process that involves the removal of components such as salts, small proteins, solvents, etc..., from the initial solution based on their molecular size by using micro-molecule permeable filters in order to obtain a pure solution.

A portable refractometer was used as a guide value during filtration, which could give a Brix-value. In general, the aim was to reduce the permeate to 0-0.5%, without a significant decrease in the Brix value in the retentate. However, the performance of the filtrations was very variable, due to the different sugar levels and the different molecular composition of the fractions. The necessary samples were analysed by means of HPLC on the sugar (and fibre) content as a check. The purified fraction was frozen at -20 ° C before being freeze-dried.

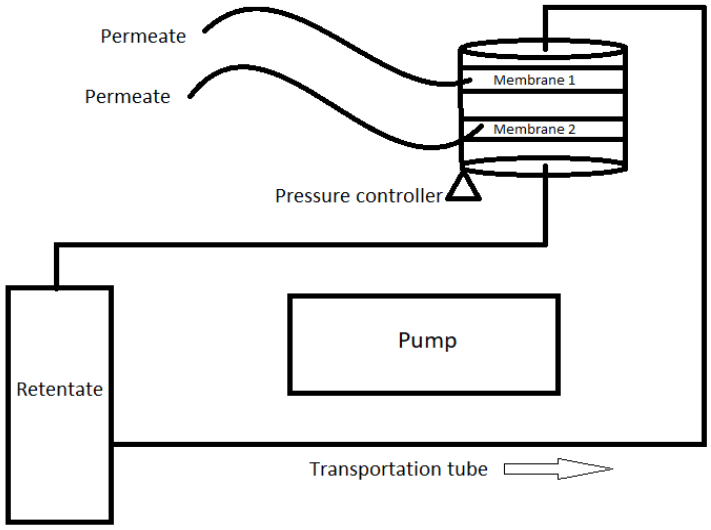

Fig. 1.1: Graphical representation of the operation of the membrane filtration installation

Insoluble fraction

The pellet obtained by water fractionation was dissolved in an equal mass of demi-water, and treated with α-amylase to convert the starch in the fraction to mono- and disaccharides. The amylase digestion occurred for 30 minutes at 70 °C (optimum effect for the used amylase) in a Thermomix at stirring speed. After the amylase digestion, the mass was immediately cooled (in a refrigerator, 4 °C) to room temperature to stop the reaction.

To separate the newly formed mono- and disaccharides from the insoluble fraction, centrifugation was carried out 3-4 times (20 min, 5000 x g) and rinsed with demineralised water. The insoluble fraction was frozen at -20 °C before freeze-drying.

Sterilisation

After freeze drying, both fractions were subjected to a sterilization by means of gamma radiation, after which it could be used in the in vitro fermentation experiments to evaluate the bioactive effect of the fibre fractions e.g. on gut microbiota composition.

Analysis

In addition to the analyses to control the purification process, analyses were carried out to obtain data on colour, moisture content, water activity (of the original powders), polyphenols, and antioxidant capacity (DPPH, ORAC). Resistant starch was also analysed, but these results will be published separately in a peer reviewed scientific paper. The data for resistant starch for the original powders however are presented in this report.

Colour, moisture content and water activity

The air-dried and ground material of each residual stream was followed for 6 months with regard to colour, moisture content and water activity, in order to get an idea of shelf life. The colour values were obtained by means of a Bench-top Colorimeter CR-5 (Konica Minolta). Values for L*, a* and b* were measured in triplicate and the colour differences were calculated relative to the values measured within 2 days of production. The formula used for this is:

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

The moisture content was measured with a moisture balance (Mettler Toledo) and the water activity with a Benchtop Water activity Meter (Aqualab).

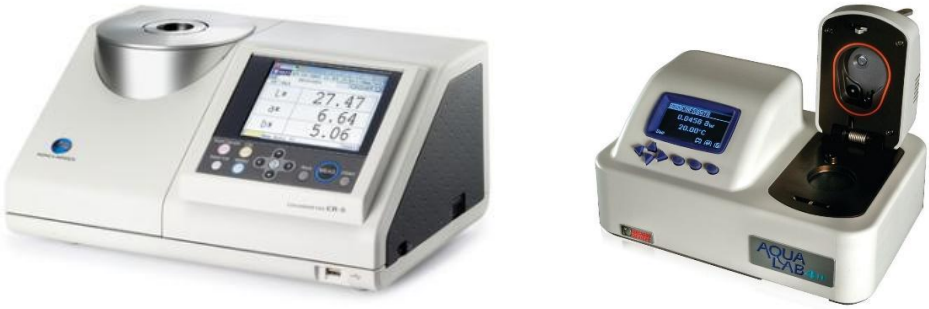

Fig. 1.2: Colorimeter CR-5 (Konica Minolta) on the left, and Water activity meter (Aqualab) on the right.

Polyphenols

All the unfractionated powders were used in a polyphenol extraction using methanol as extraction solvent. First, 0.5 g of sample was weighed in a tube. To this, 50 µl of diadzin (100 ng/µl, internal standard) and 5 ml of MeOH was added. The tubes were centrifuged (5 min, 2000 x g) and the supernatant was collected and kept at 4°C. 5 mL of MeOH/H2O (20/80%) was added to the pellets for a second extraction, followed by additional centrifugation, after which the supernatant was added to the previous one. This solution was filtered over a PVDF membrane filter (0,22 µm) in a HPLC vial for analysis.

Standard solutions were prepared to obtain different standard concentrations (0,5 to 4000 ppb), using a total of 47 phenolic compounds and an internal standard (diadzin).

Analysis was conducted using LC-MS/MS. The device used was an Acquity-Xevo TQ S, using a BEH C18 Shield RP18 (150*2.1mm, i.d. 1.7 µm) column.

Antioxidant capacity

ORAC and DPPH-assays were conducted to measure the oxygen radical absorbance capacity and free radical scavenging capacity, using a Clariometer (BMG LABTECH GmbH, Germany).

The ORAC assay was done based on the procedure as described by Bernaert et al. (2012). The samples were treated with phosphate buffer (10 mM) and a standard calibration line was made using Trolox, a vitamin E derivative. The microplate cups were loaded with 25 µl sample extract, blank solution or Trolox concentration, in addition to 150 µl of fluorescein and, after the 4th measurement cycle, 25 µl AAPH. After this, a fluorescence measurement was conducted for a period of 90 minutes at a wavelength of 520 nm. The results were calculated as are under the curve, which was used to calculate the Trolox Equivalents (TE, µmol/g DW).

The extraction procedure for the DPPH-assay was done based on the method of Zou et al (2007), with some modifications. A 4 mM 2,2-diphenyl-1-picrylhydrazyl- (DPPH-) solution was made using 100% ethanol. For the actual DPPH-assay, this solution was diluted with factor 10 to create a 0,4 mM DPPH solution. A standard calibration line was made using Trolox, a vitamin E derivative.

Sample preparation involved weighing 0.5 g of powdered sample, adding 10 mL of 70% ethanol and shaking for 2 hours at 300 rpm. After shaking, the tubes were centrifuged for 10 min at 4500 x g, 23°C. The supernatant was used to prepare different concentrations, using 70% ethanol.

After sample preparation, a clear polystyrene microplate was filled in triplicate with a blank solution (70% ethanol), standard solutions and samples, each well containing 100 µl. Subsequently, 100 µl of the DPPH solution was added to each well.

The microplate was measured for 30 minutes, with a wavelength of 520 nm. The results were expressed as absorbance and further calculations were made to obtain IC50 and TE (µmol/g DW).

Results

Pretreatment

An overview of the applied temperature and time of drying the vegetable residue streams is given in Table 1.1. The parameters were chosen based on the nature of the vegetable, with the aim of a sufficiently low moisture content (typically less than 10%) to be able to ground into a powder.

Table 1.1.: Drying temperature and time of each vegetable residue stream

Vegetable residue	Temperature	Drying time
Kale	60°C	8 h
Savoy cabbage	60°C	10 h
White cabbage	60°C	10 h
Brussels sprouts	60°C	10 h
Broccoli stems	60°C	10 h
Endive root	60°C	8 h
Chicory root	60°C	8 h
Oyster mushroom stems	60°C	8 h (largest part) and 10 h (smaller part)
Black salsify	60°C	10 h
Pointed sweet bell pepper	60°C	10 h

Fractionation and purification

Soluble fraction

For the soluble fraction after purification, control analyses were performed for protein and sugar content. Table 1.2. and Table 1.3. provide an overview of the results of these components (before and) after purification. A point of attention in the results of protein content is that the figures were calculated on the one hand in a powder and on the other in a solution, the supernatant. The results are therefore not directly comparable.

*Table 1.2: Protein content of the vegetable powders, in addition to that of the purified supernatant. * These fractions were subjected to membrane filtration in two separate parts, the first was diluted with 8L of water, while the second was not diluted. Samples taken in the retentate were therefore of different concentrations.*

	Original powder		Supernatant after purification	
Sample	**Nitrogen (g/100g)**	**Proteins (Nx6,25) (g/100g)**	**Nitrogen (g/100ml)**	**Proteins (Nx6,25) (g/100ml)**
Kale	3,48	21,75	0,08 and 0,14*	0,50 and 0,88*
Savoy cabbage	2,68	16,75	0,14	0,88
White cabbage	2,78	17,38	0,15	0,94
Brussels sprouts	3,87	24,19	0,17	1,06
Broccoli stems	3,06	19,13	0,10	0,63

	Original powder		Supernatant after purification	
Sample	**Nitrogen (g/100g)**	**Proteins (Nx6,25) (g/100g)**	**Nitrogen (g/100ml)**	**Proteins (Nx6,25) (g/100ml)**
Endive root	1,21	7,56	0,06	0,38
Chicory root	0,76	4,75	0,04	0,25
Oyster mushroom stems	1,21	7,56	0,08	0,50
Black salsify	2,54	15,88	0,07	0,44
Pointed sweet bell pepper	1,60	10,00	0,08	0,50

Table 1.3: Soluble dietary fibre and sugar content in the purified supernatant.

Sample	Soluble dietary fibre (mg/ml)	Mono- and digestible disaccharides (mg/ml)	Fibre/ sugar ratio
Kale (1)	3,25 ± 0,44	6,45 ± 0,28	0,50
Kale (2)	3,58 ± 1,00	7,21 ± 0,06	0,50
Savoy cabbage	7,59 ± 0,50	17,69 ± 0,04	0,43
White cabbage	4,07 ± 0,19	21,23 ± 0,06	0,19
Brussels sprouts	4,42 ± 0,61	19,25 ± 0,14	0,23
Broccoli stems	2,52 ± 0,03	12,26 ± 0,01	0,21
Belgian endive root	34,89 ± 0,003	12,96 ± 0,07	2,69
Chicory root	56,72 ± 0,20	5,55 ± 0,01	10,22
Oyster mushroom stems	23,07 ± 0,16	2,47 ± 0,09	9,34
Black salsify	11,00 ± 0,48	3,07 ± 0,02	3,58
Sweet bell pepper	1,77 ± 0,25	20,22 ± 0,18	0,09

In the case of Belgian endive root, chicory root, oyster mushroom and black salsify, membrane filtration was a successful medium to remove sufficient mono- and disaccharides from the remaining soluble fibre. The soluble fraction of the other residual streams contains a majority of mono- and disaccharides. An additional sugar quantification step for these four soluble fractions was carried out to evaluate their composition more accurately. From these results it could be concluded that soluble fibre was present in a factor of at least 2 (sometimes 10 or more) compared to the sugar content, and that chicory root fraction disposed of double the amount of soluble fibre compared to the other three soluble fractions.

Insoluble fraction

As verification for the insoluble fraction, the Brix-value of the supernatant was measured after every washing step. Washing was stopped when this Brix-value was less than 1%.

Analysis

Colour, moisture content and water activity

After following up each vegetable powder (not fractionated) for 6 months, the colour differences were calculated with the appropriate formula *(1). The results are presented in Table 1.4.

Table 1.4: Colour differences of vegetable powders compared to the date of production

Sample	Colour difference		
	1 month	3 months	6 months
Kale	0,79	1,12	1,50
Savoy cabbage	0,42	1,10	1,65
White cabbage	0,25	0,56	0,12
Brussels sprouts	0,71	3,06	2,94
Broccoli stems	0,56	1,85	3,25
Endive root	0,24	0,64	1,30
Chicory root	0,32	3,39	0,73
Oyster mushroom stems	0,27	2,79	1,33
Black salsify	0,77	2,72	0,61
Pointed sweet bell pepper	2,89	3,21	5,63

In general, the colour values of the vegetable powders remain relatively stable for 6 months. There are different values to be seen with white cabbage, oyster mushroom, salsify and sprouts; however, these artefacts may be caused by external factors, e.g. a different amount of ambient light during one of the measurements.

Table 1.5: Moisture levels of vegetable powders for 6 months after production

Sample	Moisture content (%)			
	0 months	1 month	3 months	6 months
Kale	7,24	8,05	7,94	7,51
Savoy cabbage	7,12	7,00	7,00	7,18
White cabbage	6,28	6,56	6,48	6,45
Brussels sprouts	5,30	5,58	5,96	5,89
Broccoli stems	6,46	7,05	7,09	7,16
Endive root	6,50	6,72	6,87	6,72
Chicory root	6,21	6,55	6,48	6,68
Oyster mushroom stems	8,84	8,98	8,88	8,62
Black salsify	9,23	9,33	9,1	8,47
Pointed sweet bell pepper	5,35	5,72	6,21	6,07

Table 1.6: Water activity of vegetable powders for 6 months after production

Sample	Water activity (A_w value)			
	0 months	1 month	3 months	6 months
Kale	0,41	0,41	0,42	0,42
Savoy cabbage	0,32	0,33	0,33	0,34
White cabbage	0,25	0,26	0,27	0,29
Brussels Sprouts	0,24	0,23	0,24	0,28
Broccoli stems	0,28	0,29	0,30	0,30
Belgian endive roots	0,35	0,34	0,34	0,35
Chicory roots	0,35	0,34	0,35	0,36
Oyster mushroom stems	0,37	0,37	0,36	0,41
Black salsify	0,48	0,48	0,47	0,48
Pointed sweet bell pepper	0,26	0,25	0,26	0,28

The results for polyphenolic compounds are shown in Figure 1.3. According to these results, endive roots and chicory roots in particular contain a higher amount of total phenolic compounds, mainly originating from chlorogenic acid ($C_{16}H_{18}O_9$).

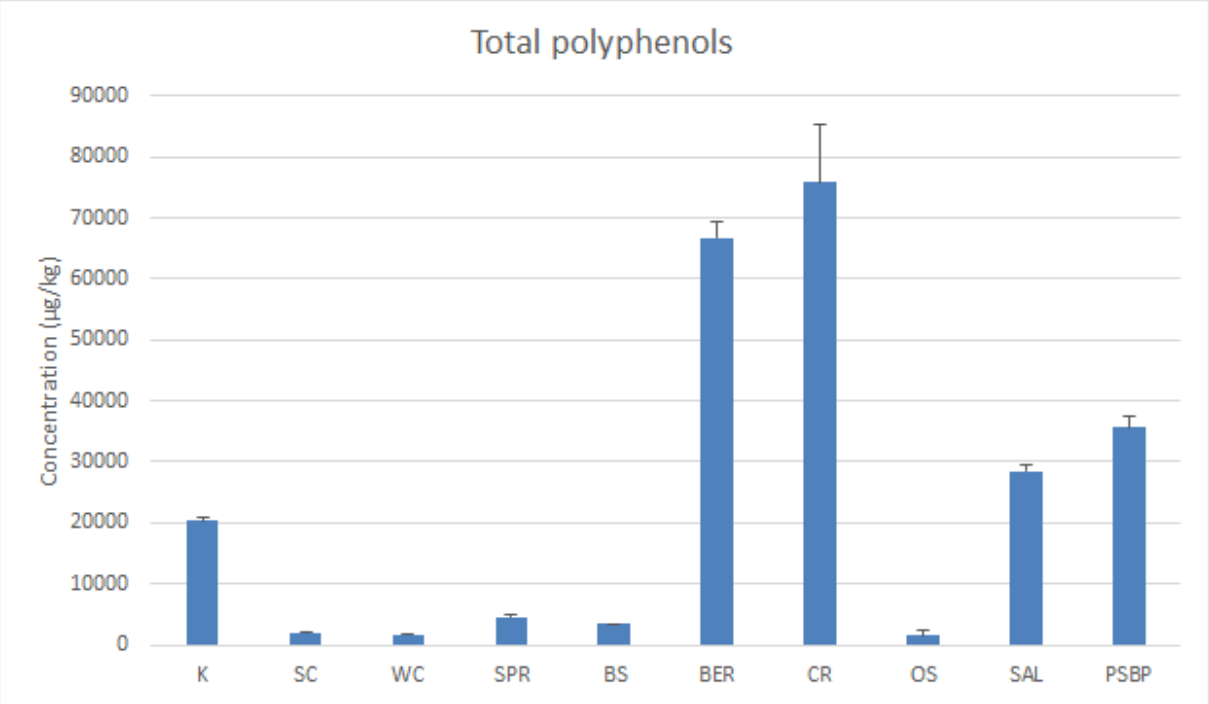

Fig. 1.3: The total polyphenol concentration in the different by-products

Resistant starch

The results for resistant and non-resistant starch are given in Table 1.7. It can be concluded that broccoli stems have the highest resistant starch content as well as non-resistant starch content, compared to the other residual vegetable streams. The streams with the lowest content of resistant starch are chicory root and Belgian endive root.

Table 1.7: Resistant starch and non-resistant starch content for the original powders derived from selected by-products.

	Original powders	
Sample	Resistant starch (g/100 g DW)	Non-resistant starch (g/100 g DW)
Kale	0,09	0,1
Savoy cabbage	0,24	0,26
White cabbage	0,35	0,38
Brussels sprouts	0,16	0,17
Broccoli stems	0,51	0,55
Belgian endive root	0,07	0,07
Chicory root	0,02	0,02
Oyster mushroom stems	0,22	0,24
Black salsify	0,08	0,08
Pointed sweet bell pepper	0,11	0,12

Antioxidant capacity

ORAC analysis

The results for the ORAC-assay are expressed in Trolox equivalents (µmol/g), and are listed in Table 1.8. and graphically shown in Figure 1.4. The antioxidant capacity is generally higher in the soluble fractions compared to the insoluble fraction, when looking at the vegetable streams that had both fractions tested (these are chicory root, Belgian endive root, oyster mushroom stems and black salsify, or the only four vegetable streams of which two useful fractions could be produced). This can bring forth the hypothesis that most of the components that dispose of antioxidant capacity present in the vegetable's streams are water soluble, e.g. polyphenols, some vitamins, etc.

It is challenging to compare these results directly to other research, due to the fact that many times a different method is used for extraction, measurement and/or processing of the data. However, it can generally be concluded that these results for antioxidant capacity are on the lower side. An explanation for this could be two things: on the one hand, the fractionation and treatment of the vegetable substrates could have had an effect on the antioxidant capacity. On the other hand, storage time (± 1 year) and therefore degradation of the bioactive components could have shown to be an important factor.

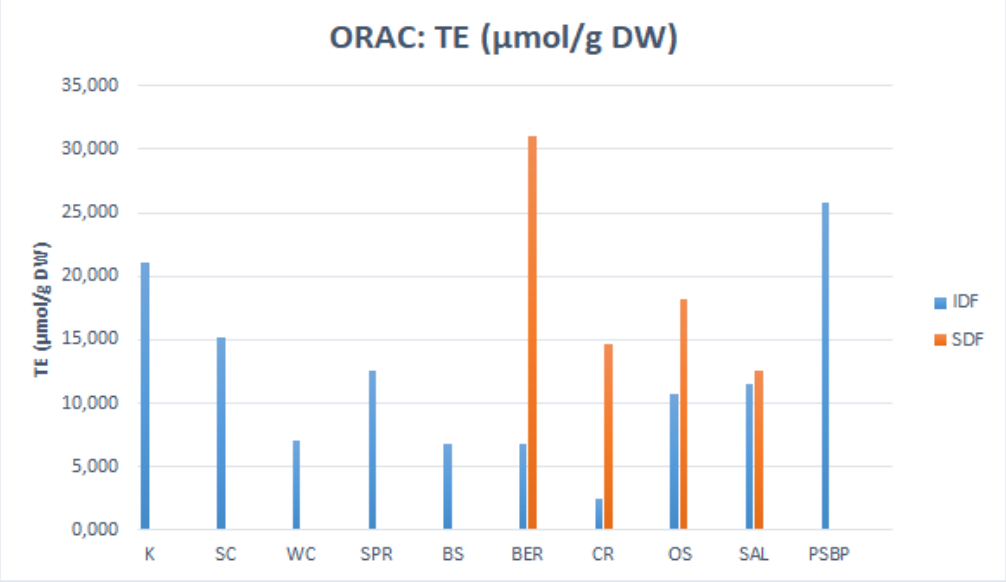

Fig. 1.4: ORAC results expressed in TE (Trolox Equivalents) for insoluble dietary fibres (IDF) and soluble dietary fibres (SDF).

DPPH analysis

The results for the DPPH-assay are given in table 1.8. and pictured in figures 1.5. and 1.6. The IC50 is the value that represents the necessary concentration of the sample to reduce 50% of the DPPH radical. In other words: the lower the IC50 value, the higher the antioxidant capacity. This antioxidant capacity can also be expressed as Trolox equivalents (TE; µmol/g DW). Note that the results from the ORAC-assay and the DPPH-assay are not directly comparable.

It can be derived from these results that fractionation of the vegetable streams has had a drastically lowering effect on the antioxidant capacity. Contrary to the results from the ORAC-assay, the SDF fractions do not seem to dispose of a higher TE-value.

Table 1.8: ORAC and DPPH results for the original powders (OR).

Substrate	Fraction	IC50 (mg/ml)	TE (µmol/gDW)
Kale	OR	2,625 ± 0,089	30,789 ± 0,369
Savoy cabbage	OR	11,621 ± 0,113	6,754 ± 0,145
White cabbage	OR	4,532 ± 0,552	17,237 ± 0,476
Brussels sprouts	OR	5,195 ± 0,488	13,646 ± 0,312
Broccoli stems	OR	5,096 ± 0,499	11,043 ± 0,092
Belgian endive root	OR	3,761 ± 0,419	20,209 ± 0,272
Chicory root	OR	6,332 ± 0,457	11,987 ± 0,072
Oyster mushroom stems	OR	Not detected	14,434 ± 1,385
Black salsify	OR	5,475 ± 0,170	12,574 ± 0,452
Pointed sweet bell pepper	OR	4,585 ± 0,559	106,161 ± 4,993

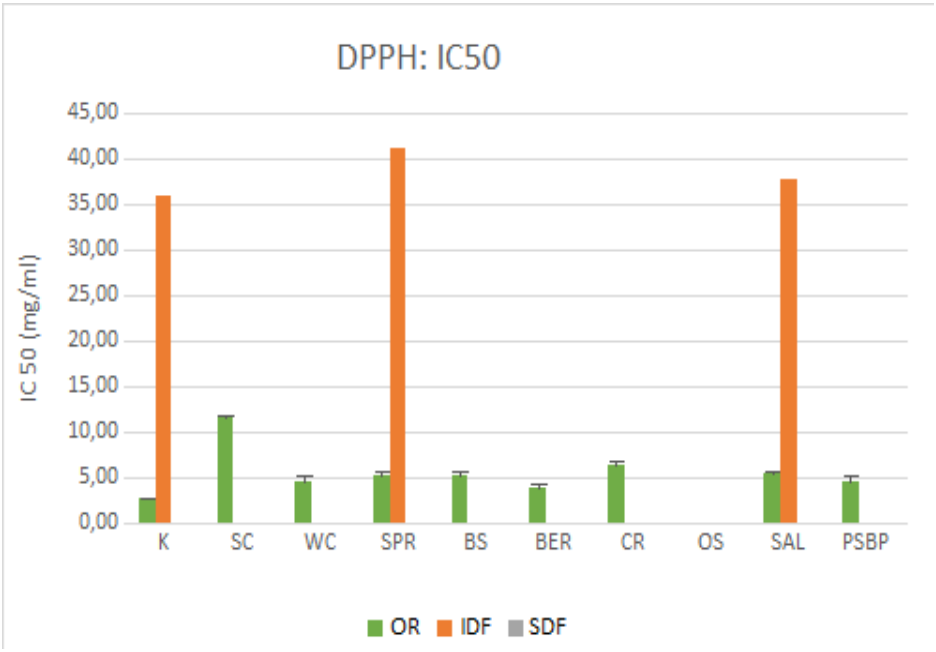

Fig. 1.5: DPPH results expressed as IC50 values for the original powders (OR), the insoluble dietary fibres (IDF) and the soluble dietary fibres (SDF)

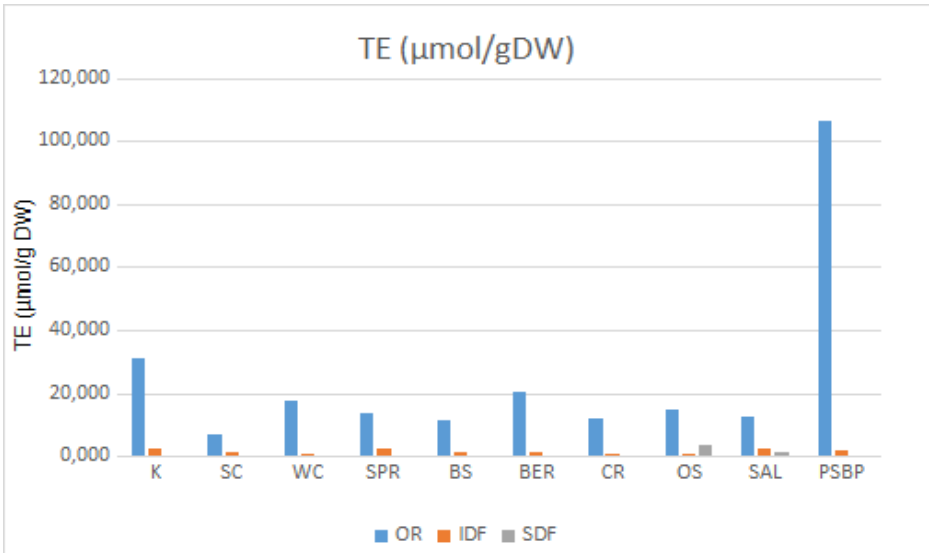

Fig. 1.6: Trolox equivalent values for the original powders (OR), the insoluble dietary fibres (IDF) and the soluble dietary fibres (SDF)

Conclusion

In this chapter, the production of a 10 various vegetable powders was evaluated with the eye on shelf-life on the one hand, and the possibility to induce a health benefit for the consumer on the other hand. Furthermore, it was proven to be possible to conduct a fractionation of these vegetable powders into soluble and insoluble fibre fractions, using a method that is relatively environmentally friendly, i.e. without using large quantities of aggressive chemicals, although a lot of water is required.

The vegetable powders, that were created by air-drying, proved to be stable during a follow-up period of 6 months, concerning colour, water activity and moisture content. This means they could be used as culinary applications in various recipes, to add colour, flavour and/or aroma, or to bring forth an enrichment of bioactive components.

Three different approaches were made to quantify the bioactivity of each powder, i.e. polyphenols, ORAC-assay and DPPH-assay. Because these three analyses are not directly relatable to each other, it is challenging to draw immediate conclusions concerning which vegetable powder disposes of the greatest bioactivity. Chicory root and Belgian endive root disposed of the highest polyphenol content, while the highest antioxidant capacity was attributed to pointed sweet bell pepper (ORAC and DPPH).

A clear conclusion that could be derived from the DPPH-assay is that the fractionation and purification of the vegetable powders resulted in a great loss of antioxidant capacity. It is therefore not optimal to use the fibre fractions with the aim of elevating bioactive potential.

The fibre fractions of the vegetable powders were produced with the intention of investigating the prebiotic potential by determining the resistant starch content and by *in vitro* experiments, conducted in a different study. The vegetable powder containing the most resistant starch proved to be broccoli stems, which could in term provide the greatest potential of all selected

Based on these results we could hypothesize that the insoluble fraction of broccoli stems would also dispose of the highest resistant starch content, attributing to this fraction the highest potential for beneficial prebiotic effects in the gut.

The soluble fibre fractions contained a relatively high amount of mono- and disaccharides, in addition to poly- and oligosaccharides (and thus fibre) can additionally serve as natural sugar-substitutes. These applications can be particularly interesting for patients that suffer from dysbiose (unbalanced microbiome flora in the gut) or other conditions such as depression, obesitas or irritable bowel syndrome. The four substrates that contained the lowest amount of sugars, i.e. chicory root, Belgian endive root, oyster mushroom stems and black salsify, have the potential to be used as functional ingredients in food products with the added benefit of fibre and/or antioxidant supplementation.

1.3.2. Press-cake from apples

Developed in collaboration at VIVES and ILVO

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Introduction

When apple juice is obtained by pressing apples, the apple juice is collected and commercialised, and a press cake residue containing fibres, outer skin and seeds is discarded. Apple juices can be obtained by either pressing the apples at a specialized processing company who has a fixed processing factory, or directly at any given locality using a mobile platform that can easily be moved to where the demand is the highest. In Belgium there is a growing trend to press apples via mobile platforms to provide the freshest possible juices and to deliver them directly to the customers. By doing so large amounts of by-products in the form of apple press-cakes are generated, but these by-products are currently not utilised for other applications and simply regarded as a waste fraction. However, these press-cakes still contain a lot of interesting compounds. Challenging is the fact that fresh press-cakes still contain a lot of water and are prone to spoilage unless stabilised. The press-cakes can be stabilized by drying and stored as such for processing at a later time. The dried press-cake can be fractionated (eliminating the seeds) and grinded into a powder and vacuum sealed for long term storage.

Aims

In this chapter we explored the application of two microwave based drying methods on the fresh press cakes from apples and investigated the nutritional and physical characteristics of the obtained apple press-cake powder.

Methods

Apple press cakes

For this chapter fresh press-cakes were obtained by VIVES from the local producer Lombarts Calville[1] in Voormezele with additional apples from other smaller producers from West-Flanders. The apples that were used were all class II discards representing a mix of Jonagold and Cox cultivars of Belgian origin. These class II by-products were characterised by aesthetic defects in size, shape and/or colour that made them unsuited for fresh market sales.

All the apples were pressed by Lombarts Calville using a continuous process with a flat-belt conveyor developed by the company VORAN. After pressing, the press-cakes were collected

[1] https://www.tuinsappen.be/

in buckets that were closed after filling to be transported at VIVES where the press-cakes were transferred to freezer bags until they could be dried. Two different drying technologies were applied: microwave-drying and vacuum-microwave-drying after which the dried press-cakes were transferred to ILVO for further analyses.

At ILVO (Melle, Belgium) the dried press-cakes were milled using a centrifugal mill (ZM 200, Retsch, Germany) with a sieve size of 0,75 mm. The samples were compared with the aim of investigating the effect of the used drying technique on the characteristics and composition of the resulting dried pulp, e.g. colour, antioxidant capacity, bioactive components and sugars.

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.

Fig.1.7: Microwave drying installation at VIVES. The same system was used for the Vacuum-microwave drying experiments

Physical characteristics

To get an idea of the macroscopic parameters of the powders, the colour was measured with a Bench-top Colorimeter CR-5 (Konica Minolta). See the chapter on Fibre extractions from a selection of 10 vegetables by-products and food waste fractions.

Further measurements for the macroscopic parameters included moisture content after milling the pulp into powder, measured with a Moisture Analyzer (Mettler Toledo), and a control measurement for water activity, which was taken using a bench top water activity meter (4TE, Aqualab).

Bioactive compounds

To investigate the bioactive properties of the apple pulp samples, they were analysed with the focus on antioxidant activity and content of polyphenols.

Concerning the antioxidant activity, two assays were applied, nl. DPPH and ORAC assays. Both analyses consisted firstly of an extraction of the sample, and secondly a photo spectrometric measurement using a microplate reader (Clariostar, BMG Labtech, Germany).

The DPPH analysis made use of Trolox as a standard solution and the reaction with DPPH, measured by absorbance. The ORAC analysis was based on the reaction of antioxidants with APPH in the presence of fluorescein, using Trolox as a standard solution and measured by fluorescence.

Fig. 1.8: Clariostar microplate reader, BMG Labtech

Polyphenol content was analysed using an extraction protocol using methanol, and measurement with LC-MS. To identify the polyphenolic compounds in the samples, 47 standards were included in the test.

Sugar analysis

A general sugar analysis was conducted to get an idea of the possible mono-, di-, or polysaccharides still present in the samples after being separated from the liquid fraction of their original form (whole apples). This was done using HPLC technique in combination with a TSK-gel column. Used standards were glucose, sucrose, fructose, raffinose, stachyose and pectin.

Results

Physical characteristics

The colour difference between the two samples was "perceptible at a glance", according to the information provided by the International Commission of Illumination (CIE).[1] In short, the sample dried with the vacuum-microwave drying technique was brighter, more red and more yellow than the sample dried with only an atmospheric microwave dryer. This can be interpreted as the vacuum-microwave dried sample being more visually acceptable towards the consumer.

Table 1.9: Lab-values and colour difference between the two samples; AP-MG = Apple pulp microwave dried; AP-VMG = Apple pulp vacuum-microwave dried.

Sample	L*	a*	b*
AP-MG	62,97	10,29	30,20
AP-VMG	63,04	12,31	32,61
Colour difference	3,15		

The moisture content of the samples after milling were 8,26% and 8,67% for the microwave dried apple pulp (AP-MG) and vacuum-microwave dried apple pulp (AP-VMG), respectively. The water activity was 0,43 (AP-MG) and 0,44 (AP-VMG), while at the moment of transfer from Vives to ILVO, the values were 0,26 for both samples. A possible explanation for these elevated values could be exposure to air, thus resulting in the absorption of humidity by hygroscopic effect. Since the values were very low, both powders will be microbiological stable. A water activity higher than 0,6 creates a more suitable environment for the growth of microorganisms, which means that the product is microbiologically unstable or unsafe.

Fig. 1.9: Apple pulp, microwave dried and milled (left) and Apple pulp, vacuum-microwave dried and milled (right).

[1] http://zschuessler.github.io/DeltaE/learn/

Bioactive compounds

The results of the DPPH analysis are shown in Table 1.10. From these results it can be concluded that the vacuum-microwaved sample disposes of the highest TEAC or Trolox Equivalent Antioxidant Capacity, the lowest IC50 value and the highest value for Trolox equivalent. This was to be expected, as the addition of a vacuum to the microwave-drying technique leads to a shorter drying time.

Table 1.10: Results from DPPH-assay of apple pulp samples.

Sample	IC50 (mg/ml)
AP-MG	14,28 ± 2,99
AP-VMG	7,99 ± 0,38

Table 1.11: Results from ORAC-assay of apple pulp samples

Sample	TE (µmol/gDW)
AP-MG	12,46 ± 2,24
AP-VMG	16,88 ± 4,55

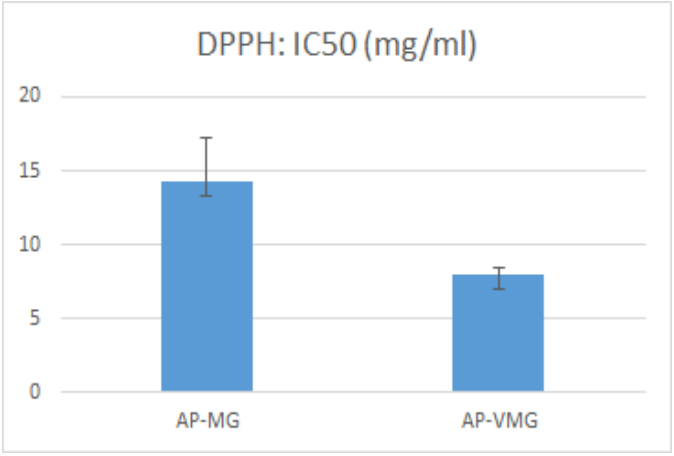

Fig. 1.10: DPPH results expressed IC50 values for apple press cakes that were dried with the classic microwave drying method (AP-MG) and with the vacuum drying technique (AP-VMG).

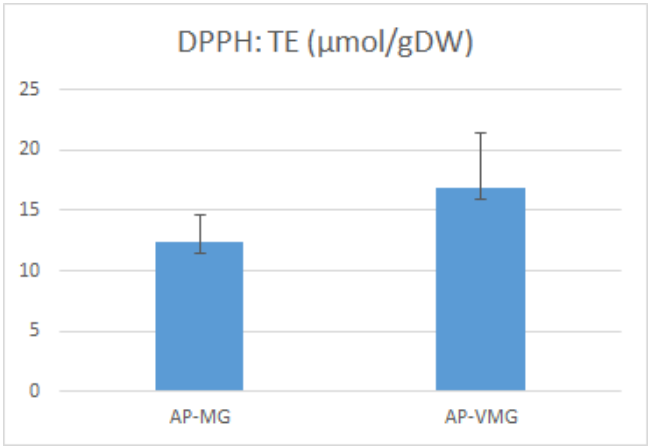

Fig 1.11: DPPH results expressed as Trolox equivalents for apple press cakes that were dried with the classic microwave drying method (AP-MG) and with the vacuum drying technique (AP-VMG).

The results from the ORAC-assay show similar Trolox equivalent-values for the microwave and the microwave-vacuum technique (Table 1.12).

Table 1.12: Results from ORAC-analysis of apple pulp samples.

	TE (µmol/g)
AP-MG	85,45 ± 6,89
AP-VMG	82,49 ± 1,77

Polyphenols

The results for polyphenolic compounds for microwave-dried press cake from apples (AP-MG) and vacuum-microwave-dried press cake from apples (AP-VMG) are shown in figures 1.12 and 1.13. Most of the concentration values for polyphenolic compounds are comparable. AP-VMG produces slightly fewer total polyphenols than AP-MG. This could be due to quercetin and chlorogenic acid polyphenols, which both have high concentration values and strong differences between both groups, and thus also influence the total values.

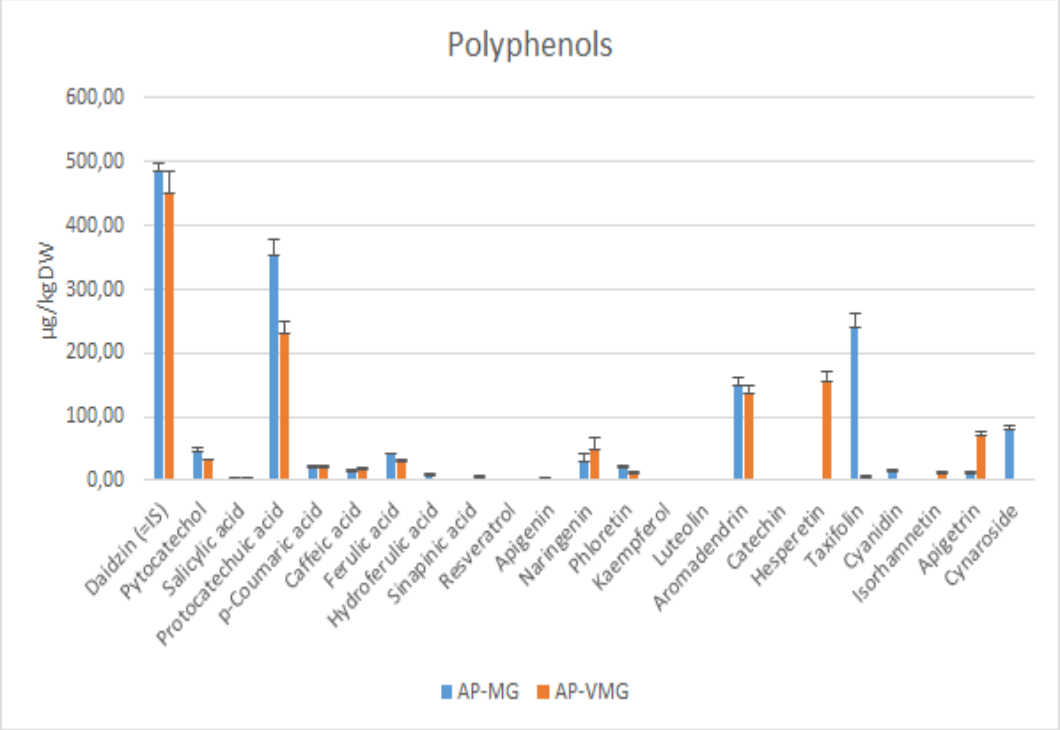

Fig. 1.12: Results for 0-1000 µg/kgDW of the concerning polyphenol in apple press cake samples.

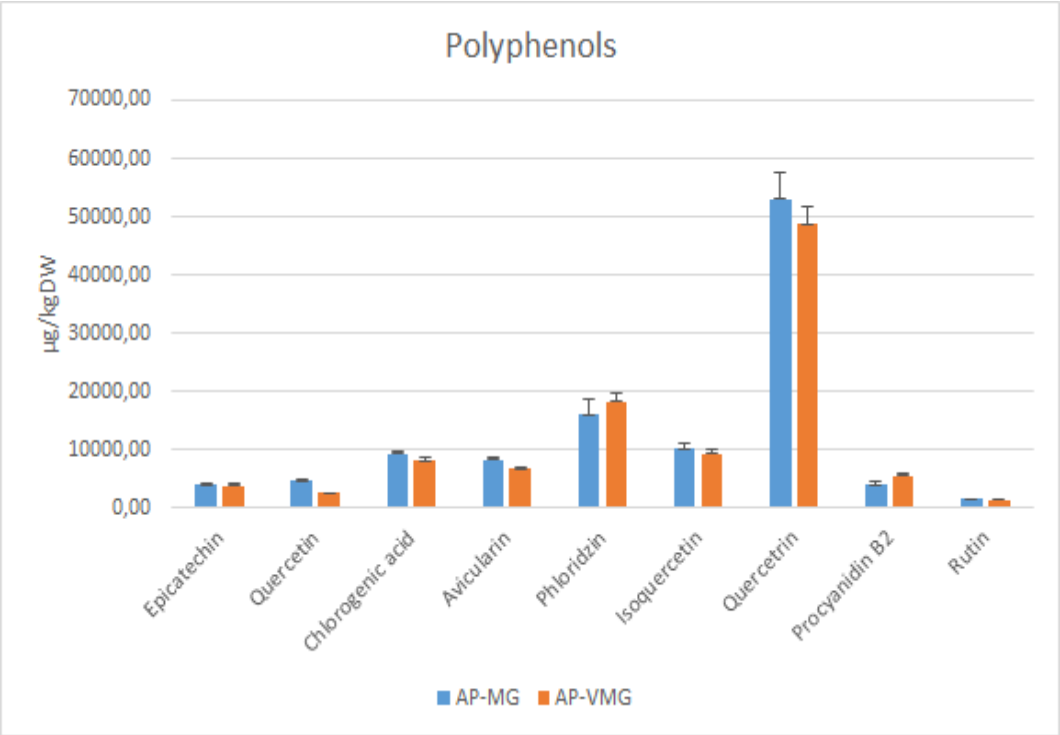

Fig. 1.13: Results for more than 1000 µg/kgDW of the concerning polyphenol in apple press cake samples.

Sugar analysis

A general sugar analysis is shown in Table 1.13 There are more di- and monosaccharides in the vacuum-microwave-dried press cake from apples (Glucose, Fructose, Sucrose). However, there is more pectin present in the traditionally produced microwave-dried press cake from apples. No raffinose of stachyose were detected in both groups.

Table 1.13: Sucrose, Glucose, Fructose, Pectin, Raffinose and Stachyose concentrations are shown per selected groups. Abbreviations: AP-MG: microwave-dried press cake from apples, AP-VMG: vacuum-microwave-dried press cake from apples. Cells with a slash indicate that the polyphenols concentrations were below the detection limits, ppm: parts per million

Sugars	Concentration (mg/g)	
	AP-MG	AP-VMG
Sucrose	1,15 ± 0,13	1,49 ± 0,08
Glucose	0,89 ± 0,02	0,93 ± 0,21
Fructose	2,27 ± 0,09	2,45 ± 0,28
Pectine	0,56 ± 0,005	0,36 ± 0,003
Raffinose	-	-
Stachyose	-	-

Discussion

Microwave drying of by-products presents a number of advantages over classic air-drying or cryogenic drying.

Air drying has the disadvantage that the product needs to be spread out in either a thin layer or distributed over small fragments to maximise the contact surface with the surrounding atmosphere. Contact surface with the warm surrounding airflow is necessary to stimulate evaporation of the water contained within the product. However, air-drying has the second disadvantage that the product tends to form a crust around the product that may complicate the further drying as the dried exterior might form a barrier to the water vapor and prevent its escape from the product. This effect is off course strongly dependent on the matrix that is being dried, but can strongly lower the efficiency of the drying process.

The advantage of microwave drying is that the product is heated from the inside, contrary to air-drying where it is heated from the outside there is no formation of a crust around exposed parts of the product. Microwave drying leads to a volumetric heating which means that all of the product can be heated to the desired temperature at the same time.

The most noticeable difference in the samples between the two drying techniques is in the colour of the dried product. With a calculated colour difference index of 3,15 the two samples are located on the boundary between ‘slightly noticeable colour difference’ and ‘visible colour difference’. This means that samples that were dried using different microwave drying techniques can be easily distinguished by the naked eye, although the difference remains subtle. The results of the ORAC and DPPH analysis seem contradictory, but comparing these results directly with each other is tricky since they both use a different approach to assess the antioxidative potential of compounds within the samples. The analysis of polyphenols for both samples shows that the polyphenol content of the samples is virtually identical, with the exception of hesperetin that is present in the vacuum dried sample, but absent in the other sample, and taxifolin which is present in the non-vacuum dried sample but absent in the vacuum dried one.

In conclusion no major difference in phenolic compounds and antioxidant capacity was observed between the tested drying methods. Therefore, any of these methods can be selected for dehydration of apple press cakes for industrial purposes. Microwave drying at an industrial scale has the advantage that it is a time and/or energy efficient technique compared to other more traditional drying techniques such as hot air-drying, sun drying or freeze drying.

1.3.3. Aquafaba: Discovering future uses for residual cooking fluids from the chickpea and soy-industry

Developed at ILVO

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Introduction

Currently several companies in Flanders produce food products of which one of the ingredients are peas from Fabaceae (leguminous plant family), such as soybeans (*Glycine max*) and chickpeas (*Cicer arietinum*) or produce canned chickpeas (Greenyard[1]). During the production these companies often heat up these peas or beans by cooking them in water or they add protective liquid in the cans before canning. In most cases the residual water streams used to cook the products is considered a waste product and subsequently discarded. However, they could actually be used as an important vegetable protein source, which could serve as a meat substitute for vegans. For example, in the last couple of years, an amount of chickpeas cooking fluid (also known as “Aquafaba”) is able to serve as a meat substitute specifically for vegans, as it causes foaming effects during stirring/mixing, so that it can act as a substitute for animal-produced whippable proteins such as egg white. As such, there are many “Aquafaba” recipes found on the internet, to produce “vegan” chocolate mousse, chocolate donuts, meringue and even mayonnaise.

However, although “Aquafaba” seems to be popular on several internet forums and groups, the product itself cannot be readily found in the supermarket or bought as a finished product. On most internet sources, “Aquafaba” is described as the moisture found in cans of chickpeas, which are available in the supermarket. This liquid can be whipped similar to egg white. The reason why the “Aquafaba” moisture cannot be found as a product in itself, can presumably be because the moisture contains (next to the amounts of some proteins) some amounts of the glycoside class molecules, called saponins, which are also very soluble in water. These saponins are chemical components having an amphiphilic effect, which means that they can actually be a reason why the moisture is able to exhibit the "whipping" effect.

Saponins

Saponins are chemical molecules produced by various plant species, but also by lower marine animals and some bacteria. These amphipathic glycosides have a soap-like effect and produce foam when shaken in aqueous solutions. Structurally they have one or more hydrophilic glycoside moieties (such as saccharides), which is combined with a lipophilic triterpene or

[1] https://www.greenyard.group/

steroid derivative. Different types of saccharide bonds are possible, as well as different types of sapogenins (triterpenoid and steroidal) (Riguera 1997, Francis et al. 2002).

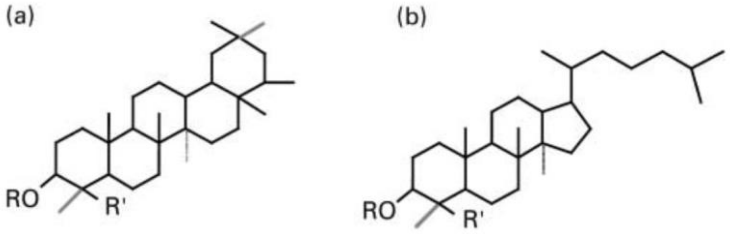

Fig. 1.14: Basic structures of sapogenins: a triterpenoid (a) and a steroid (b). Source: Francis et al., 2002.

These saponins may however have negative effects on the health of consumers, making it essential to be able to check the quantity of saponins before products derived from residual streams are placed on the market.

Toxicity of saponins

Saponins are generally dissolvable in water, and are known to be poisonous to fish and insects. They however appear to be almost non-toxic to man. Saponins appear in large numbers in small amounts in a large variety of plants, but only 28 of them are commonly eaten by man. Especially soybeans, chickpeas peanuts and spinach clearly contain saponins and appear to be safe (Oakenfull 1981). On the other hand, there appear to be some opinions (Question No EFSA-Q-2005-221) about saponins in *Mudhacu longifolia* L. on the European Food Safety Authority (EFSA), as they could be undesirable substances in animal feed. These saponins can cause toxic effects, but have been shown to cause beneficial health effects as well. They have, as glycosides, low oral bioavailability, but can be hydrolysed in the intestine and cause systemic toxicity. Toxicity studies and observations of toxic effects in feeding studies were reported and crude total saponins or defatted seed meal from various *Madhuca* species were used. Mice were used for these studies, and the oral LD50 in mice of crude *Madhuca* saponins was about 1.0 g/kg body weight. On EFSA, no comparable studies for human food have been shown. One study however did do an evaluation of Quillaia extract (E999) which contains saponins, and gave it and acceptable daily intake (ADI) of 3mg spray-dried extract/kg body weight (bw) per day [1]. It would therefore be wise to investigate the amount of saponins present in residual streams (cooking water) and to check whether these amounts are not higher than those present in edible beans.

Aims

Measure the amount of saponins found into residual streams (cooking fluid) derived from several Flemish food companies, and compare these values with the quantities found in cooked (and therefore edible) samples of chickpeas and soybeans. If the quantities and

[1] doi: 10.2903/j.efsa.2019.5622

concentrations are not higher than those found in edible legumes, taking into account the volumes consumed, the cooking fluid is considered safe and can thus be used as a vegetable protein source and replacement for egg and can as such be safely sold as “Aquafaba”.

The results of this chapter will be submitted for publication in a peer-reviewed scientific journal. For this reason, this chapter presents a shortened version of the research output that was generated by ILVO until the research article is officially published.

Methods

We have used extraction and spectrophotometric methods, based on previously published articles (Hiai et al., 1976; Le, 2018), to quantify the total amount of saponins, present within several freeze-dried samples of chickpeas, soy and quinoa.

Results

Saponin-concentration of chickpea samples available in the supermarket were measured, more specifically that of saponins in uncooked chickpeas, edible cooked chickpeas and canned chickpeas. Additionally, the saponin content of the cooking fluid, produced by boiling beans and that inside commercially available cans was measured. Lastly, we measured the saponin concentration present in the chickpea cooking fluid produced by the Flemish company ‘The Hobbit’.

In a similar way the saponins-concentrations were measured in uncooked soybeans available in the supermarket, the cooked soybeans version and the cooking fluid. Furthermore, the amount of saponins present in the cooking fluid, also produced by the Flemish company ‘Hobbit’, was analysed for its saponin content.

Discussion

All cooking fluids can be considered safe to eat. The measured concentration of saponins were always lower than those present in the edible cooked chickpeas/soybeans samples. It did not matter if the samples were cooked in the kitchen, in an industrial plant or kept in a liquid food can for a long period. Saponin concentrations were always lower in the cooking fluid, compared to the edible vegetable samples.

We also measured transfer of the saponin molecules from the chickpeas/soybeans to the liquid after cooking, by comparing the saponins concentrations of all samples in dry masses. As expected, based on the measured results, these reduction levels were not that high (all below 40%). Although saponins are water soluble molecules, the largest part (> 50%) still remains in the cooked edible vegetables, which is of course positive news for the potential use of these cooking fluids in vegan recipes.

1.3.4. Pointed Sweet Bell Pepper

Developed at ILVO

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Introduction

Pointed sweet bell peppers are descendants of peppers from South and Central America and were introduced to Europe by Spanish and Portuguese explorers in the 15th and 16th centuries. The sweet peppers are believed by the majority of experts to have been first cultivated in Italy, but its origins are mostly unknown although some experts hypothesise Cuba as a possible point of origin. Red pointed sweet bell peppers were introduced to the United States in 1932, and today they are exported to countries around the world, mainly from the Dominican Republic. Red pointed sweet bell peppers can now be found on a smaller scale at local farmers markets throughout Europe, the Caribbean, and the United States.

Red Pointed sweet bell peppers are botanically classified as the species *Capsicum annuum* var. longum and are the mature versions of a sweet pepper that belongs to the *Solanaceae* or nightshade family. Also known as Cubanella, Friarelli, or Aji Cubanela, red pointed sweet bell peppers have a very mild heat, ranging 100-1000 SHU on the Scoville scale, and are most well-known as an Italian frying pepper or cooking pepper. Pointed sweet bell peppers are used in both their immature green and mature red state and are favoured for their sweet taste and thick flesh. The peppers are a staple ingredient in Cuban, Puerto Rican, Italian, and Dominican cuisine and are also utilized in both fresh and cooked applications. Pointed sweet bell peppers are an excellent source of vitamin C, which is an antioxidant that can help build collagen in the body and boost the immune system. The peppers also contain some potassium, vitamin A, folate, manganese, and vitamin K.

Availability of pointed sweet bell peppers and their by-products

Red pointed sweet bell peppers are available in the summer through early fall. Even so, they are still not as popular on the Belgium fresh market as the more classic and very commonly sold common bell pepper that can be purchased either as a green, yellow, orange or red bell pepper. However, for buyers that live outside of areas with Italian or Caribbean influence, they can be hard to find in stores and in Belgium they can be purchased in selected stores, but are not readily available everywhere.

The pointed sweet bell peppers (red and yellow) that we are considering here for BioBoost for the valorisation of their food waste fraction are in fact discards (unprocessed whole peppers) from the quality control and sorting. These peppers have the same general quality and taste as the ones that are being sold on the fresh market, but are deemed unfit for sale because

they either have esthetical defects (spots, dents, visible signs of rubbing) or are deformed to such extent that they are judged unappealing to potential buyers. The availability of these residual flows is therefore subjected to considerable variations in amounts and quality, depending on the supply and the quality of the initial produce. The seasonality of the peppers also strongly influences the availability of the subsequent fractions.

Fig. 1.15: The class II Pointed sweet bell peppers that were used for the creation of novel processed products such as the pointed bell pepper ketchup and chutney.

Applications and cooking

Pointed sweet bell peppers are well-suited to cook with, and especially as stuffed peppers. They tend to taste a bit sweeter in comparison to the very popular classic bell peppers but are characterised by thinner fleshy walls, making them suited for a more diverse range of recipes and preparations. In general appearance they are more elongated and have a more wrinkled skin than the classic bell peppers.

Aims

We seek to develop a semi-automated process to cut the pointed bell peppers mechanically and to apply a sorting step to remove the seed core, the seeds and the stems without or with minimal human intervention. The goal is to obtain a semi-finished product with minimal manual handling during the pre-processing steps. The pre-processed diced and cleaned peppers can then serve as a base ingredient for the further development of recipes and food applications.

Methods

Pointed sweet bell peppers

The pointed sweet bell peppers that were used for the tests were class II by-products with visual defects (spots, discolorations, wrinkled skin, …) (Fig. 1.15) or an unusual shape and were acquired as fresh products prior to testing at ILVO Food Pilot.

Preliminary tests

Prior to the tests in the food pilot, a small-scale lab test was performed to assess the inner structure and the abundance and implantation of seeds, and to test if seeds could easily be separated from the remaining flesh by means of buoyancy or sedimentation. The weight share of the seed core and stem was determined and seeds were submerged in water for approximately 15 minutes to determine their sedimentation properties.

Pre-processing of pointed sweet bell peppers

To determine what equipment and settings are necessary to obtain pointed bell pepper blocks of workable size and clean them from seeds and other unwanted parts (= stems and seed cores), a test was performed in the ILVO Food Pilot using an industrial Dicer (TREIF cube cutter model Argon, Maschinenbau GmbH, Oberlahr, Germany) and a Retsch® analytical sieve shaker (AS 200, Retsch, Haan, Germany) equipped with three vertically stacked sieves with screen openings of 6,3 mm; 5,6 mm and 4 mm from top to bottom respectively.

Dicing the pointed sweet bell peppers

Three different settings (without fixed blades, and blades separated by 16 or 30 mm) were used to cut the peppers into small pieces using the TREIF Dicer. The dicer has a set of fixed blades and one rotary blade. By adjusting the space between the fixed blades and the rotation speed of the vertically rotating cutting blade, blocks of different sizes can be obtained.

Fig. 1.16: The set-up for the Dicer with the fixed blades in the centre forming a square mesh and the single rotary blade below that cuts the long juliennes into cubes after they passed the fixed blades. ©Treif[1]

The class II bell peppers are placed within a loading area of the machine in which a piston is activated that pushes the peppers through the fixed blades that are positioned under a 90° angle to each other to obtain cube shaped sections (juliennes). After the fixed blades a single vertically placed rotary blade rotates at a set speed to cut the juliennes into blocks. The blocks are then transported onto a conveyor belt and collected in a recipient for further processing.

[1] https://www.treif.de/en/products/

By removing the fixed blades ring sections can be cut, while not using the rotary blade cuts the pointed bell peppers into juliennes.

Sieving the bell peppers

The diced pointed sweet bell peppers still contained pieces of stem, seed cores and seeds that needed to be removed from the flesh. The blocks obtained from the dicer were loaded onto a Retsch® analytical sieve shaker (Vibration frequency 3000 min^{-1}; vibration amplitude of 2 mm). Sieving was done twice, first dry, then with water being sprayed on the top of the sieve.

Results

Preliminary results

Seed cores and stems

Pointed sweet bell peppers and the more classic square shaped sweet pepper differ substantially in shape and internal structure. The very elongated pointed sweet bell peppers have seed strips (typically 2 or 3) that run from the top of the head all the way to the bottom. Although most seeds are located near the head where the massive seed core is located, seeds can be found along the full length of the seed attachment sites on the strips. Therefore, cutting off the head of the pepper alone is insufficient to remove all seeds. On top of that the practice of cutting off the head also causes significant losses in usable volume of the pepper's meat. The seeds are however not the only part of the pepper that needs to be removed before processing, there is also a hard and bulky stem that needs to be cut off somehow.

Fig. 1.17: A series of pointed sweet bell peppers showing the great variety in shape and size between peppers (left) and a pointed sweet bell pepper that was cut in half to show the location of the seed core (A), the seeds that are mainly located near the seed core (B) and other seeds attached along the seed strips that run along the full length of the peppers (C).

Although manual labour is preferred because of the maximum efficiency in separating the comestible meat from the unwanted parts (stem, seed core and seeds), this method quickly becomes too intensive and time consuming when upscaling the processing capacity to industrial levels. Interestingly, some lessons can be learned from the processing of square bell peppers for which industrial processes are already in place and widely applied. Several methods exist, but most of them still require manual intervention for the successful separation of diced parts. Either the initial steps involve the manual removal of the stem and seed core, or the peppers are cut automatically without discrimination for unwanted parts and later on processed on a conveyor belt where workers hand-pick the undesired parts. A more modern approach would involve the use of an automated camera- and expulsion system to remove unwanted parts based on their colour (seed cores are whitish-yellow and stems are green while the flesh is red or yellow), but this would also require a considerable investment and could only be justified for large scale processing plants with high throughput. Such installations do not yet exist in Belgium at the moment.

Losses by cutting off the tip of the pepper

An 'quick and easy' method to get rid of the stem, the seed core and the majority of the seeds is to simply cut off the head of the pointed sweet bell pepper. Removing the top 4 cm of the peppers head will in most cases be enough to remove the entirety of the seed core, however the great diversity of sizes and shapes within the pointed sweet bell peppers can pose an additional difficulty to correctly estimate the amount of the peppers head that requires removal. Here both manual removal with a sharp knife or machinal removal by inserting the peppers onto a loading area where converging cutting discs will automatically do the cutting at a predetermined distance. The machinal removal could however be problematic because of the highly variable shape of the peppers, especially for class II discards that usually are far more bent or twisted compared to class I peppers.

Fig.1.18: Manually processing pointed sweet bell peppers is the most efficient method for cleaning the peppers while minimising waste fractions (left) but requires manual labour and is time consuming. Pointed sweet bell peppers with remaining seeds (middle) and manually washed pointed bell pepper (right).

Seed buoyancy in aqueous medium

Seeds from pointed sweet bell peppers and square bell peppers were collected and placed in a glass of water to observe the seeds buoyancy to verify if seeds could be separated from the remaining pieces of processed pepper by submerging them in water. Seeds have an irregular shape and vary in size between 3 and 4 mm at their longest diameter.

It was observed that micro-bubbles of air are formed around the fresh seeds when immersed into water, thus initially improving buoyancy. Upon stirring the water with the seeds, a large portion of seeds sinks to the bottom, but there are at all times seeds that remain floating while others completely sink to the bottom. Curiously a minority of seeds seem to remain somewhat in the middle of the solution. Over time more seeds tend to sink, but there is not a clean trend to be observed. Therefore, buoyancy cannot be used to efficiently get rid of the seeds by placing the processed peppers in a water solution.

Additionally, the submergence in water would result in a loss of bell pepper sap that is leaching from the diced pieces of pepper flesh. This would impact the taste of the product.

Removing the stem

In the first step the pointed sweet bell peppers are cleaned in water to remove all remains of dirt, dust or other debris that sticks to the outer skin. In some pointed sweet bell peppers, there is a strong accumulation of dirt between the stem and the head of the peppers. This is however strongly dependent on the shape of the peppers and highly variable. Cutting off the tip of the pepper and removing manually the leftover pieces of flesh revealed that there was often dirt accumulation between the stem and the top of the pepper, which would require an extra step to thoroughly clean.

To minimise manual labour and remove the stems and seeds cores automatically, the unprocessed peppers could also be mechanically cut in small pieces and unwanted parts would then subsequently be removed by automated camera detection systems.

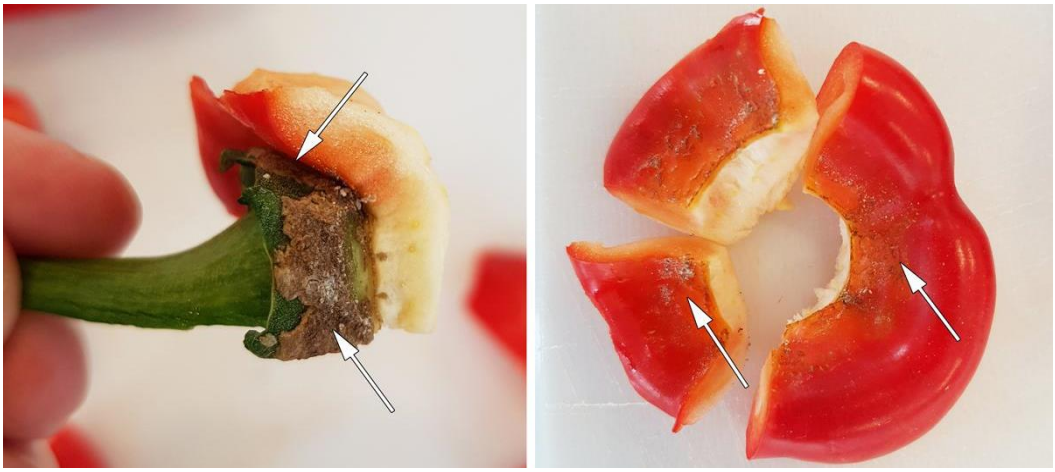

Fig. 1.19: Arrows indicate the accumulation of dirt and debris against the stem of the pepper.

Dicing the pointed bell peppers into blocks

The pointed sweet bell peppers were then loaded unprocessed into the dicer and cut into small pieces, the result is a mix of pointed sweet bell pepper blocks, seeds and stems (Fig. 1.21 D).

Fig. 1.20: The cube cutter dicer that was used for the experiments at ILVO Food Pilot.

The machine settings that offered the best results in terms of cube size was with the fixed blades separated by 16 mm. Seed cores remained most intact when using the 30 mm grid blades or without the fixed blades. The smallest cut at 16 mm proved to be the best choice. The smaller blocks also allow for a higher efficiency in packing with less intermediate air pockets in between the pieces of pepper.

Removing stems and seed cores from the batch

In the following step these undesired parts need to be removed from the batch. This can be done either by implementing a manual sorting step where operators will pick out the unwanted parts on sight, or as is now often done in large scale processing units by automated camera or laser sorting. The advantage of automated optical sorting systems is that the selection algorithm can be specifically programmed to detect only the green parts (= stems) and white parts (= seed cores) and remove them, for example with a high-pressure air-blow system. Freefall systems where the items are being detected and removed while being projected off a high-speed conveyor belt are particularly well-suited to deal with small sized blocks such as the diced pointed sweet bell peppers.

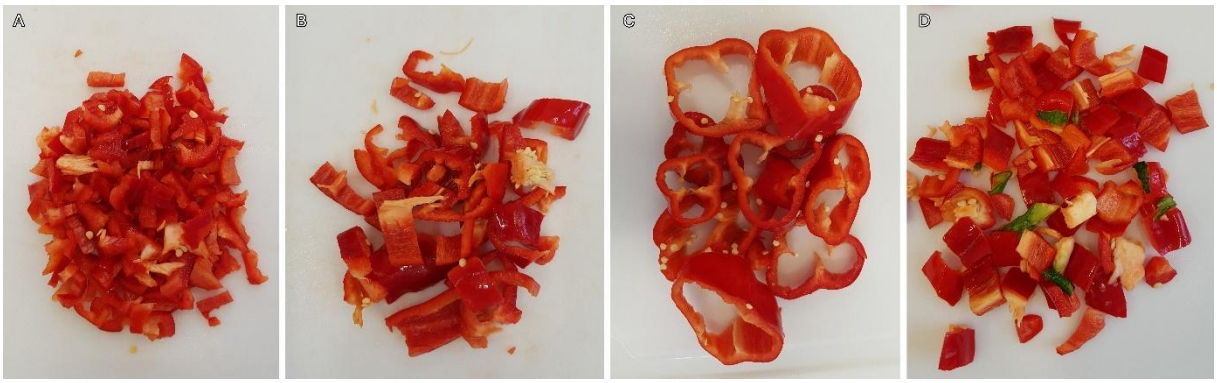

Fig. 1.21: Four ways of cutting the bell peppers have been applied. Left (A) with fixed blade 16 mm separated, middle (B) with fixed blades 30 mm separated and right (C) without fixed blades (only rotary blade for cutting) and (D) with fixed blades of 16 mm but cat without cutting off the tip with the stem. The remaining seeds that are attached to the remains of the seed cores or that stick to the cut pieces are clearly visible and must be removed in a next step.

Removing the seeds

Bell pepper seeds are not toxic, but have a bitter taste, harder texture than the sweet bell pepper fruit flesh and are considered unpalatable by many people. The remaining seeds that are detached but still stick to the diced peppers need to be removed. Placing the pointed sweet bell peppers on a sieve with a mesh size of 4 mm which is the maximum size of the seeds at their longest edge (2-4 mm) allows them to effectively get rid of all the remaining seeds. It was noted that with the block size that was used (blades separated by 16 mm), small pieces of pointed sweet bell peppers (about 40% of the total mass) still managed to pass through the sieve with grid sizes of 6,3 and 5,6 mm. The 4 mm sieve blocked almost all pieces of pointed sweet bell peppers, with almost negligible losses.

This sieving step can either be done dry or by addition of water to flush out the seeds. The dry method may be preferred as it avoids the addition of water to the product and the loss of juice that would otherwise be washed out. On the counter side, the dry method may take longer as the seeds tend to stick to the wet pieces of pointed sweet bell peppers and some seeds may still remain stuck even after a longer period of sieving. The effects of different sieving methods should be investigated in more detail when upscaling the processes.

Fig. 1.22: Sieving over a 4mm grid (left) allows to eliminate all remaining seeds when sprayed with a water spray at 5 bar pressure (right).

Proposed solution for semi-automatic processing of pointed sweet bell peppers

Although manual cutting and seed/core removal produces the most qualitative results, an automated or semi-automated alternative is possible. The most efficient system is when the raw product is processed as a whole without a preliminary manual step to cut off or remove the stem and seed core. Undesired pieces that end up in the diced product such as seed cores and stems can be removed by an automated camera detection and expulsion system. Several techniques already exist and are extensively used in the vegetable processing industry. These camera systems usually provide satisfying results. However, one of the culprits in processing raw bell peppers are the small seeds that tend to stick into the pieces of cut peppers and must be removed before further processing. Sieving provides a means to get rid of the seeds, which could be recuperated as a pure by-product of their own for other applications. Dry-sieving proved difficult but might be feasible under specific conditions that exceeded the scope of the current research. However, a wet washing of the pieces of pointed bell pepper in the sieving system was sufficient to remove all remaining seeds but presents additional concerns in terms of water usage and microbial load. Additionally, a part of the juice that is released upon cutting will inevitably be washed away, probably affecting the taste perception of the processed peppers.

1.3.5. Innovative pre-processing techniques of edible Brassica for the development of a minimally processed purée.

Developed at ILVO

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Introduction

In this chapter we explore the pre-processing of by-products from two edible *Brassica* species, Brussels sprouts (*Brassica oleracea* var. gemmifera) and kale (*Brassica oleracea* var. sabellica), to obtain a minimally processed purée that contains a maximum of health promoting compounds and with maximal retention of organoleptic qualities. This product form was chosen since it can be used in a wide variety of preparations and convenience foods that are already well known and often consumed in Belgium.

Brussels sprouts and kale both originated from cultivated *Brassicaceae* that have been selected to obtain unique cultivars to fit particular culinary demands. Although the concentrations and composition of nutritional components may be different between varieties or cultivars, their positive effects on human health remains unaltered. In this chapter we consider two source fractions originating from the harvesting, selection and processing of Brussels sprouts and kale. The by-products used for this research are crops that were discarded for aesthetic reasons during the quality control for fresh market applications (class II products).

Understanding the influence of processing on the microstructure of these products will help to design food products with improved organoleptic parameters such as flavour and texture. The objective of the current research is the study of quality and chemical parameters of Brussels sprout and kale purée produced by different heating methods.

Brussels sprouts

Brussels sprouts are native to the cooler regions of northern Europe and have developed from the wild cabbage varieties. It is believed to have originated in Belgium, hence the name, where it was cultivated from the nineteenth century onwards. With its rising popularity its cultivation has spread to other European countries, including the UK where its production is carried out on a relatively large scale. In the USA it is mainly cultivated in California and in Asia it is very common in China and South-Korea.

Brussels sprout is a tall and single-stemmed cabbage in which many compact tiny buds develop at the base of the leaves along the entire tall central stalk of the Brussels sprouts plant. Cultivars have been selected to provide a spread in harvests and later-maturing ones can withstand temperatures down to -10 °C. Good quality Brussels sprout should be about 1-

2,5 cm in diameter (i.e., 1-1,5 cm in Europe and 2,5 cm in North America), have a fresh appearance, bright green, closed outer leaves, and should be firm. It also has to be sweet and tender with bright green leaflets and a clean, white cut where they have been trimmed from the stalk. The buds should be tight and firm; loose, split leaflets or elongation of the central stem indicate over maturity and are discarded for sale. Yellowing indicates advanced senescence and loss of quality, while wilted or puffy sprouts tend to be woody in texture and have off-flavours.

The edible parts which include axillary buds are either consumed raw or cooked by boiling. Most consumers use the product fresh or frozen, and as a pot-herb. Processing is primarily by freezing, although a small proportion of the crop is canned. The leaves and other edible tissues of Brussels sprouts are good sources of many potentially protective dietary components.

Kale

Kale (*Brassica oleracea* var. sabellica*)*, sometimes also referred to as leaf cabbage, is a cultivar of cabbage and in fact the same species as Brussels sprouts, although kale is cultivated for its large green edible leaves. Ornamental varieties also exist, with central leaves that can be white or purple in appearance. Kale originated in the eastern Mediterranean and Asia Minor, where it was cultivated as a food plant as early as 2000 BC. Curly-leaved varieties already existed along with flat-leaved varieties in Greece in the 4th century BC. These varieties, which were referred to by the Roman kale, are considered to be the ancestors of modern kales.

Kale is an annual plant grown from seeds. It is considered to be very hardy and frost resistant, making it thrive in wintertime. It is well-known that a cold period also influences the taste of the leaves, making them sweeter after the plant has been exposed to frost.

Water activity and moisture content

Water is the main component in most fresh plant-derived food materials and the water content of fresh fruits and vegetables is typically very high (around 80%).

Moisture content is the amount of water in food, taken as a key indicator for the maturity, density, viscosity, state, stability, and important for the quality and processing characteristics of food products. High moisture content is related to microbial, enzymatic and chemical reactions which could have an effect on the quality and shelf-life of plant-derived food materials.

A_w is the availability of water in a food and excludes moisture that is bound and unavailable for the microorganisms to use. It is one of the most critical factors in determining quality and safety of foods. Water activity affects the shelf life, safety, texture, flavour, and smell of foods.

Bioactive compounds

Consumers become increasingly aware of the need for a continuous supply of components from plants to enjoy optimal health benefits. As a result, there is an increasing tendency from

mainstream consumers to demand high quality products that can provide a higher added value (Cartea et al. 2011). In this context the popularity of cultured *Brassicaceae* crops is rapidly increasing due to their high nutritional value (rich sources of vitamins, minerals, fibres, nutritional acids, sugars, carotenoids, polyphenols and plethora of secondary metabolites). These crops are known to have a positive impact on the health and wellbeing of humans by regulating digestion processes, supplying slow-release sugars, reducing blood pressure, affecting uptake and metabolism of fats, preventing diabetes, cardiovascular diseases and possibly by delaying the aging processes. Data about vitamins, minerals as well as the nutritional components of the main vegetable cultured Brassica's discussed below is based on the information in Table 1.14 provided by the USDA National Nutrient Database for Standard Reference.[1]

Table 1.14 shows that among cultured *Brassicaceae*, Brussels sprouts and kale are the richest in phytochemical components. They are also rich in vitamin C and carotenoids. Also, in cultured *Brassicaceae* products, there is a high amount of folate (vitamin B9 or folacin) that reduces the risks for vascular diseases, cancer and neural tube defects. Among green leafy vegetables, kale and Brussels sprouts are important mineral sources that accumulate high levels of P, S, Cl, Ca, Fe, Sr and K, those play important roles in different metabolic processes. (Kim et al. 2009).

Organic acids

Organic acids are a group of organic compounds containing carboxylic groups. In solution, organic acids release protons, which determine their acid taste. They are widely distributed in fruits and vegetables, originated from biochemical processes or some microorganisms' activity, such as yeasts and bacteria (Hernandez et al., 2009). Among the organic acids found in plants, those from the tricarboxylic acid (Krebs) cycle, namely citric, acetic, succinic, ascorbic, maleic, fumaric, malic and oxaloacetic acids, can be distinguished. All these acids occur in catalytic amounts in plant tissues, although only citric and malic acids are regularly accumulated (Harborne et al., 1999).

Such compounds are also extensively used as additives in food industry, namely as antioxidants (tartaric, malic, and citric), acidulants (tartaric, malic, citric, and ascorbic acids), or preservatives (sorbic and benzoic acids). Malic and citric acids are the most abundant organic acids in cultured *Brassicaceae*. Malic acid performs various functions, such as acting as a substrate for mitochondrial adenosine 5'-triphosphate (ATP) production, providing nicotinamide adenine dinucleotide (NADH) to the cytosol, maintaining the cytosolic pH value and acting as an osmoticum and as a counterion for potassium or sodium. Citric acid has an

[1] https://ndb.nal.usda.gov

important role in the translocation of iron in the roots and its long-distance transport through the xylem to the leaves.

Table 1.14: Edible and nutrient content of 100 grams of edible cultured Brassicaceae vegetables (USDA 2015).

		Kale	Brussels sprouts	Broccoli	Cabbage
	Water (g)	84.04	86.00	89.30	92.18
	Energy (kcal)	49	43	34	25
	Protein (g)	4.28	3.38	2.82	1.28
	Total lipids (g)	0.93	0.30	0.37	0.10
	Carbohydrate (g)	8.75	8.95	6.64	5.80
	Fibres (g)	3.36	3.8	2.6	2.5
	Sugars (g)	2.26	2.20	1.70	3.20
Minerals	Ca (mg)	150	42	47	40
	Fe (mg)	1.47	1.40	0.73	0,47
	Mg (mg)	47	23	21	12
	P (mg)	92	69	66	26
	K (mg)	491	389	316	170
	Na (mg)	38	25	33	18
	Zn (mg)	0.56	0.42	0.41	0.18
Vitamines	Vit. C (mg)	120	85	89.2	36.6
	Thiamin (mg)	0.110	0.139	0.071	0.061
	Riboflavin (mg)	0.130	0.090	0.117	0.040
	Niacin (mg)	1	0.745	0.639	0.234
	B-6 (mg)	0.271	0.219	0.175	0.124
	Folate (µg)	141	61	63	43
	Vit. A (µg)	500	38	31	5
	Vit. E (µg)	1.54	0.88	0.780	0.15
	Vit. K (µg)	704.8	177	101.6	76

Glucosinolates

Glucosinolates (GLS) are the main class of secondary metabolites found in cultured Brassicaceae vegetables and their presence in the genus cultured Brassicaceae (Brussels sprouts and cabbages included) is of major concern in any consideration of their effect in animal feeds and human foods (Fenwick et al., 1983).

Glucosinolate contents of Brassicaceae are influenced by environmental factors such as soil, climate and cultivation conditions including fertilization, harvest time, and plant position. However, wide genetic variations in the contents and composition of glucosinolates have been reported from previous studies (Verkerk et al. 2009). Brussels sprouts and kale are mainly characterized by glucoiberin, sinigrin, progoitrin, but also glucoraphanin, and gluconapin (Cartea and Velasco, 2008).

Among all cultured Brassicaceae vegetables, Brussels sprouts have the highest level of total glucosinolates with the predominant components being sinigrin, gluconapin, progoitrin, glucotropaeolin, glucobrassicin, and glucoerucin (Heaney and Fenwick 1980; Kushad et al.

1999). Sinigrin, glucobrassicin, and glucoiberin are identified as the predominant glucosinolates in cabbage and kale (Nilsson et al., 2006; Cartea et al., 2008). They are mainly amino acid-derived secondary metabolites responsible for the characteristic flavour and odour of these vegetables and are believed to have defence functions to the plants

Thermal treatment by steam cooking, microwaving, and stir-frying don't induce significant changes in the contents of glucosinolates. During industrial processing of Brassica vegetables, especially during canning, the thermal treatment can affect GLS levels considerably. Oerlemans et al., (2006) described thermal degradation of individual GLSs in red cabbage. Degradation of all the identified GLSs occurred when heated at temperatures above 100°C. The indole GLSs 4-hydroxy-glucobrassicin and 4-methoxyglucobrassicin appeared to be most susceptible to thermal degradation, even at temperatures below 100°C. Canning, the most severe heat treatment, will result in substantial thermal degradation (73%) of the total amount of GLSs.

However, boiling is more effective in reducing the levels of glucosinolates (approximately 90%), by leaching into the cooking water. Boiling or steaming for 10 min was able to reduce sinigrin by 9.6 and 29.1% in cauliflower (Girgin and El, 2015). Steaming for 10 min, boiling for 15 min, and high-pressure cooking for 7 min imply losses between 20-33% and 45-60% in pressure treatment and boiled vegetables, respectively. Breakdown products of aliphatic glucosinolates increased from 5 to 12% in steamed, 18 to 23% in pressure-cooked, and 37 to 45% in boiled samples (Vieites-Outes et al., 2016).

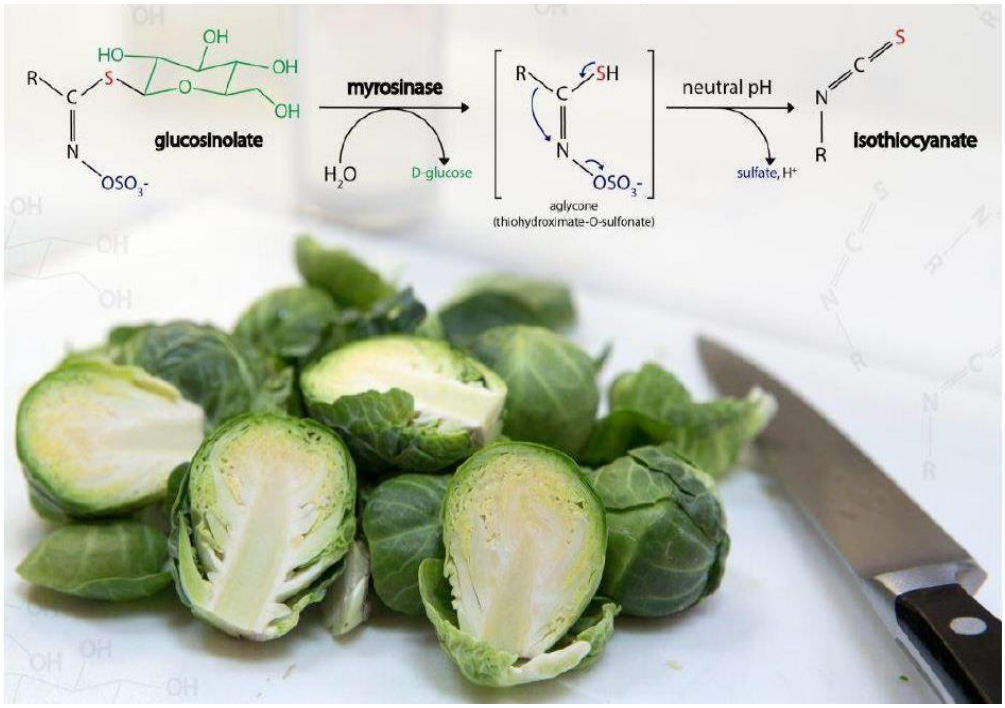

Figure 1.23: Schematic overview of the enzymatic breakdown of glucosinolates in Brussels sprouts Source: https://www.hsph.harvard.edu/nutritionsource/2016/11/21/science-of-flavor-cruciferous-vegetables-brussels-sprouts/

The health-promoting effects of cultured Brassicaceae vegetables have been attributed to the physiological properties of glucosinolate breakdown products, mainly isothiocyanates and indoles. These compounds have been reported to act as anticarcinogens during many stages of cancer development through multiple mechanisms, including activation of detoxification enzymes (phase II enzymes), inhibition of carcinogen activation enzymes (phase I enzymes), antiproliferation, blocking cell cycle, and promoting apoptosis, among others (Hayes et al. 2008).

Thermal inactivation of myrosinase: Effect of boiling and blanching

The presence or not of active myrosinase directly impacts the degradation of glucosinolates. To consume intact glucosinolates, the inactivation of myrosinase is a key point during food processing. By boiling vegetables belonging to the *Brassicaceae* family, the glucosinolate myrosinase system is altered by the partial inactivation of myrosinase and loss of myrosinase in the cooking water. In addition, there will also be a loss of enzymatic cofactors such as the electrostatic potential (ESP). The duration and temperature during cooking determine how large the loss / inactivation will be.

The source from which the myrosinase originates determines its heat stability. When extracting and purifying myrosinase from broccoli as a powder that is heated, it appears that the myrosinase is inactivated at ≥40 ° C. After heating at 60 °C for 3 minutes at pH 6,5 this type of myrosinase loses about 90% of its activity. With homogenized red or white cabbage, heating for 30 minutes at pH 7,0 and 70 °C will be required to reduce the activity of the myrosinase enzyme by more than 90%. The optimum temperature for the myrosinase activity from broccoli appears to be 30 °C. This temperature is clearly lower than the optimum temperature for the myrosinase from red and white cabbage that is around 60 °C. For Brussels sprouts this is 50 °C. The higher thermostability of plant myrosinase reported in cabbage and Brussels sprouts, compared to broccoli, can be partially clouded by the effect of heat on plant cell disruption [1].

Blanching whole Brussels sprouts at 95 ° C for 10 minutes or steam blanching at 105 ° C for 5 minutes will cause complete inactivation of the plant myrosinase. Glucosinolate concentrations are reduced by 13% or 3% respectively through these treatments. Although the reduction of glucosinolates is usually the result of leaking after boiling in water, the decrease in myrosinase activity of plants is positively related to the blanching temperature. It is clear that myrosinase from different plants has a different thermostability and is inactivated at a different temperature.

[1] https://www.cambridge.org/core/journals/proceedings-of-the-nutrition-society/article/effect-of-cooking-brassica-vegetables-on-the-subsequent-hydrolysis-and-metabolic-fate-of-glucosinolates/ 30841862B9D531DF37CDAF97853EF8DC

Chlorophyll

Like other dark green vegetables, many cruciferous vegetables are rich in chlorophyll. It is the main pigment in green vegetables and the primary compound in photosynthesis. Chlorophyll molecules are conjugated tetrapyrroles, to which a cyclopentanone ring, conjoint with ring III, has been added. The macrocycle is planar. All naturally occurring chlorophylls have a propionic acid residue at position 17. The position 173 is generally esterified with long chain alcohol, usually phytol. Chlorophyll b differs from chlorophyll a by the presence of an aldehyde residue instead of a methyl group at position 7 (Figure 1.24).

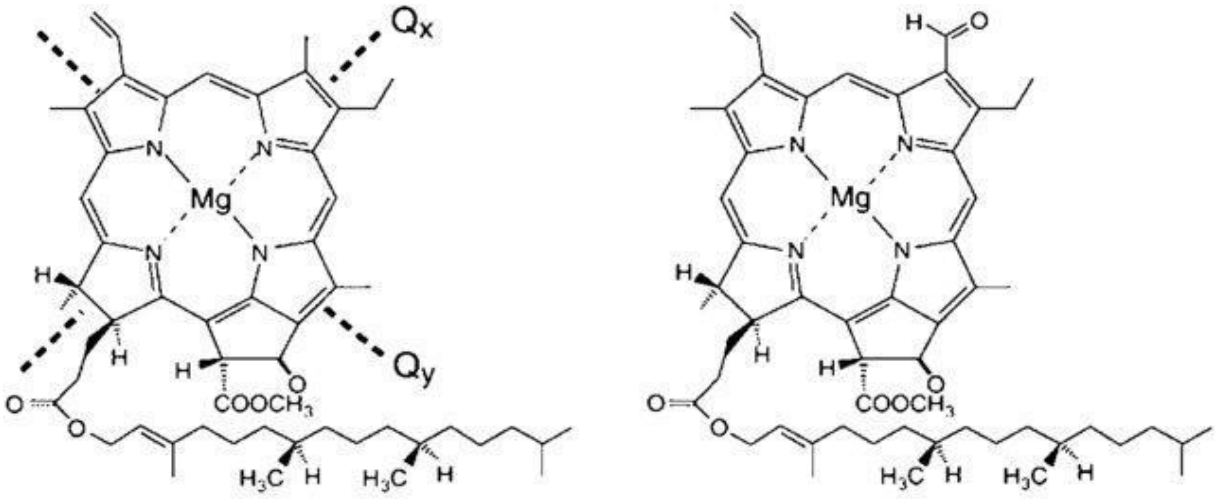

Fig. 1.24: The molecular structure of (A) chlorophyll a and (B) chlorophyll b. Source: Chen and Blankenship (2011).

The chlorophyll molecule is made up of a hydrophilic part, the macrocycle, and by a hydrophobic part, the phytol chain. The most hydrophilic segment of the macrocycle is the cyclopentanone ring and the propionic ester group (position 17). Therefore, non-esterified macrocycles are much more polar than esterified ones. Chlorophylls and related molecules present a closed circuit of conjugated double bonds, which allows them to absorb light at slightly different wavelengths. Several studies have shown chlorophyll's important health-promoting functions such as prevention of carcinogenesis or inhibition of various mutagens (Egner et al. 2001, 2003; Smith et al. 2001).

Consumers choose vegetables based on appearance. An important parameter in this subjective selection criteria is colour which is affected by chemical, biochemical, microbiological and physical changes that occur during development, maturation and post-harvest handling. Colour is also used as a factor to evaluate the processing conditions impact on the final product. Colour is the basis for sorting many products into commercial grades, it relates more directly to consumer perception of appearance and subsequently its buying decision.

Several colour coordinate systems can describe the colour of an object (Clydesdale, 1978; Francis, 1980; Hunter and Harold, 1987). One of the most popular systems is CIE (Commission

Internationale de l'Eclairage) L* a* b* (red, green and blue), which is used here. Chemical bonds absorb light energy at specific wavelengths, therefore compositional information can be determined from spectra measured by spectrophotometers. Within the visible wavelength range, the major absorbers are the pigments such as chlorophylls and other coloured compounds. In this regard, for vegetable products, such as kale purée, it is well known that the green colour is correlated with the chlorophyll content (Sant'Anna et al., 2013). They are highly susceptible to degradation during processing conditions resulting in food colour changes.

Chlorophyll retention is related to the preservation of chlorophyll-binding proteins, as well as inhibition of the catabolism of thylakoid lipids, since chlorophyll degradation is the first step in thylakoid degradation. During processing operations, such as chopping, cooking or freezing of plant material, released intracellular acids and enzymes contact with chlorophyll-protein complexes. This contact, combined with the physical damage to the plant tissue, initiates chlorophyll degradation.

During thermal processing, the central magnesium atom of the porphyrin ring of chlorophyll is replaced by two hydrogen atoms to form pheophytin, which is accompanied by an undesirable colour change from bright green to olive brown (Mackinney and Joslyn, 1941; Gold and Weckel, 1959; Schwartz and Lorenzo, 1990). Similarly, enzymatically generated chlorophyllide converts to pheophorbide under the influence of heat.

Brassica flavour and sensory characteristics

Flavour and sensory characteristics are an integral part of the quality parameters of fresh-cut and processed vegetables. The main enzymes that are involved in flavour, colour, and texture of fresh cut vegetables are peroxidase (POD) and polyphenol oxidase (PPO). PPO is widely distributed in vegetables and is believed to cause their main flavour changes, that most of the time, causes the decreasing of products marketability. This enzyme catalyses the oxidative reaction associated with undesirable browning of damaged tissues in fresh fruits and vegetables, by oxidizing polyphenols to quinones, in the presence of oxygen. The quinones may condense and react non-enzymatically with other phenolic compounds, amino acids, proteins and other cellular constituents to produce brown melanoidin pigments (Rouet-Mayer et al. 1990).

There have been many studies on methods for controlling vegetable browning, such as: thermal processing (blanching and pasteurization), ultrasound, high pressure processing and pulse electric fields. POD enzymes play a role in degrading food quality including undesirable flavour and colour changes.

Influence of processing

Brussels sprouts are mostly grown outdoors and harvested seasonally, which makes freezing an important preservation technique for extending their commercial availability throughout

the year. The quality attributes like texture, flavour and nutrients content can change during frozen storage of untreated vegetables, due to the action of enzymes, which remain active even at temperatures below 0 °C. That's the reason why processing of frozen vegetables embraces several preliminary operations to prepare the final product, such as selection, washing, peeling and cutting, blanching and/or other pre-freezing treatments, cooling, freezing and frozen storage.

The usual technique to inactivate enzymes in vegetables is blanching. It consists of heating at high temperature (generally in water at 85–100 °C or with steam, less frequently with microwaves, radiofrequency or infrared radiation). Short times of exposure are enough to reduce the incidence of degradation reactions during storage. Since blanching is a heat treatment, changes associated with thermal processing can be expected. Heating can reduce the concentration of bioactive compounds in the plant tissue through thermal breakdown and leaching. It can also inhibit enzyme activity and increase the extractability of some compounds by disrupting the plant cell wall matrix so that the compounds become more bioavailable.

Aims

The aim of this research is to quantify and describe several physical and chemical characteristics of a processed *Brassica* purée. In doing so, the optimum processing conditions can be identified. The chosen conditions play a role in the quality of the product and also the storage conditions and time can be optimised. This knowledge is helpful in producing a high-quality product out of by-products or class-II of the *Brassica* that are otherwise discarded from the fresh market.

Methods

Purées preparation and processing

Brussels sprout purée preparation

To be able to carry out analyses, samples are first prepared from fresh vegetables, treated on a lab-scale in different ways to make purée. The Brussels sprouts were obtained from the fresh market or the auction and were cultivated in Belgium or the Netherlands. The different treatments that were performed in Brussels sprouts are shown in Table 1.15.

Table 1.15. Brussels sprouts sample preparation.

Sample	Treatment
1-2	blanched in water
3-4	cooked in water
5-6	raw
7-8	cooked in steam
9-10	blanched in steam

Cooking sprouts was done for 15 minutes, while blanching was done for 5 minutes. After the heat treatment, the Brussels sprouts were mixed. The final product was stored in falcon tubes and frozen at -40°C.

Kale purée preparation

Kale purée processing with VaculIQ®

A total of 100 kg of kale was processed with different treatments. The kale used in the experiments was obtained from the fresh market or the auction and were cultivated in Belgium or the Netherlands. The first purée prepared was the F^- sample, used as the reference. This sample was frozen at the beginning and then it was cut with the K64AC8 vacuum cooking cutter (Seydelmann - vacuum cooking cutter K64AC8 Diksmuide - Belgium) at 6011 rpm for 300 seconds with an end T°C of -1,2°C, and stored in the freezer at -40°C.

Fig. 1.25: Kale source material (left), after cuttered (middle) and prepared for blanching (right).

In the next step the raw sample was used to prepare purée with the last step under vacuum (VACULIQ® 1000. VaculIQ® GmbH & Co. KG. D-46499 Hamminkeln - Germany) at room temperature. The mash was fed with a slight vacuum (0-1 bar) to the delivery point of the effective vacuum in the system. The combination of the generated vacuum and the pressure force generated by the rotating spiral within the filter compartment drew the liquid phase through the holes of the filter sieve, while the solid phase was stuck to the side of a cochlea that carried it to the exit. Thus, the system allowed to separate solid matrix and the liquid one. These were analysed separately. Figure 1.27. and 1.28. show steps that were used to make the other samples.

Fig.1.26: Kale samples during the processing. A: Kale sample cuttered after blanching, B: sample B(L), C: sample F after cuttering, D: sample F after VacuIIQ® pressing treatment, sample F+H after VacuIIQ® pressing treatment, F: sample F+H when coming out of the VacuIIQ®.

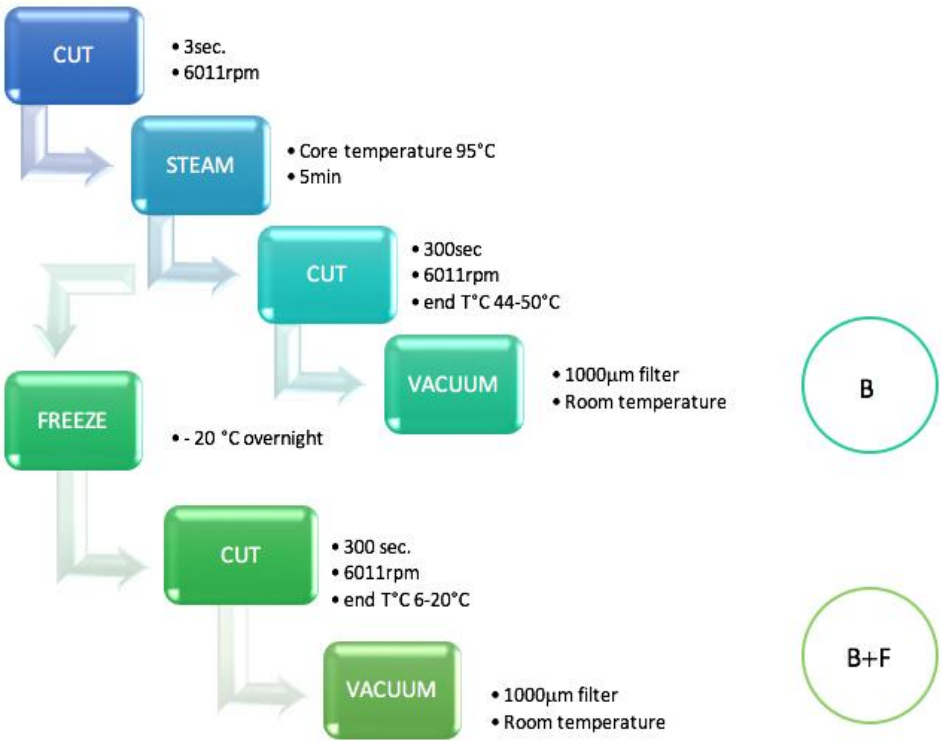

Fig. 1.27: Sample B and B+F preparation.

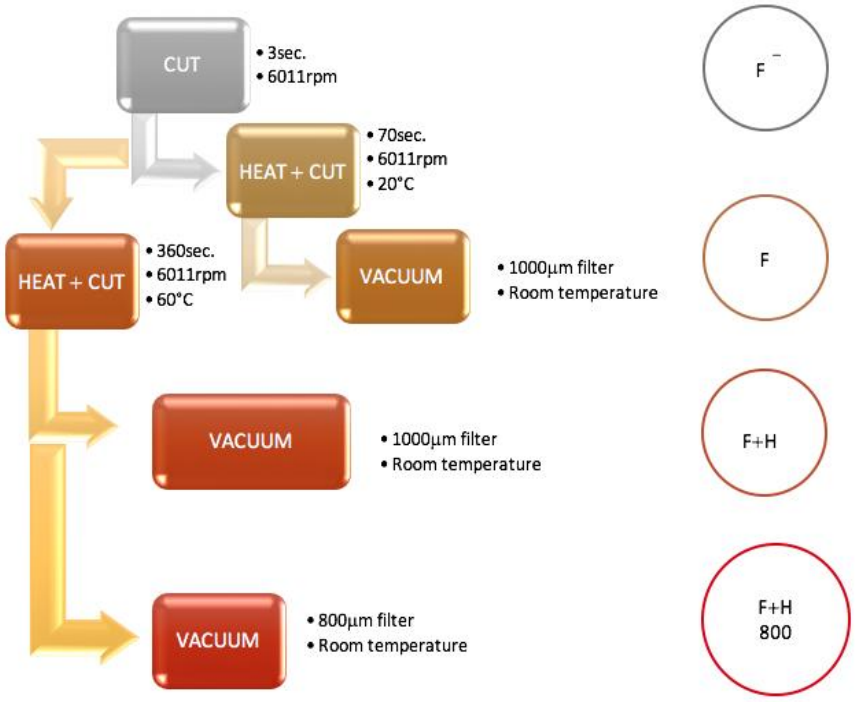

Fig 1.28: Sample F, F+H and F+H 800 preparation.

Samples can be distinguished from each other by the cooking treatment. B samples were blanched in steam while F samples were heated at 20°C or 60°C by using the Sydelmann - vacuum cooking cutter.

Kale processed with autoclave

A total of 7 kg of kale was processed in the autoclave, that was the last cooking treatment, used as sterilization.

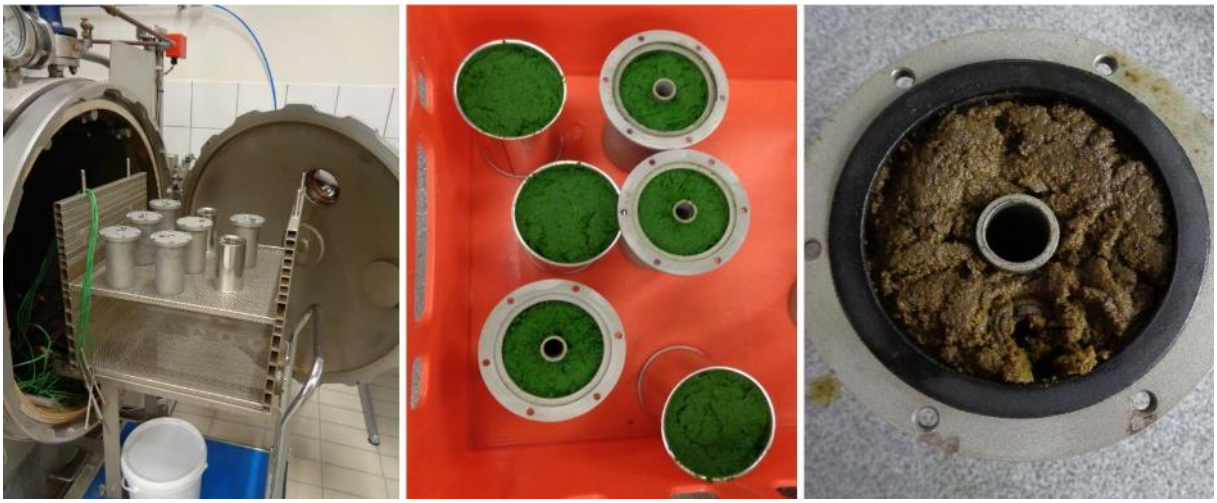

Fig. 1.29: Processing of samples with the industrial autoclave. The autoclave set-up (left), the samples prior to autoclave treatment (middle) and a sample after autoclave treatment with a typical brownish colouration (right).

The first purée prepared was the S sample, used as the reference, in the same way, that was used to prepare the previous samples. The proceeding is shown in figure 1.30.

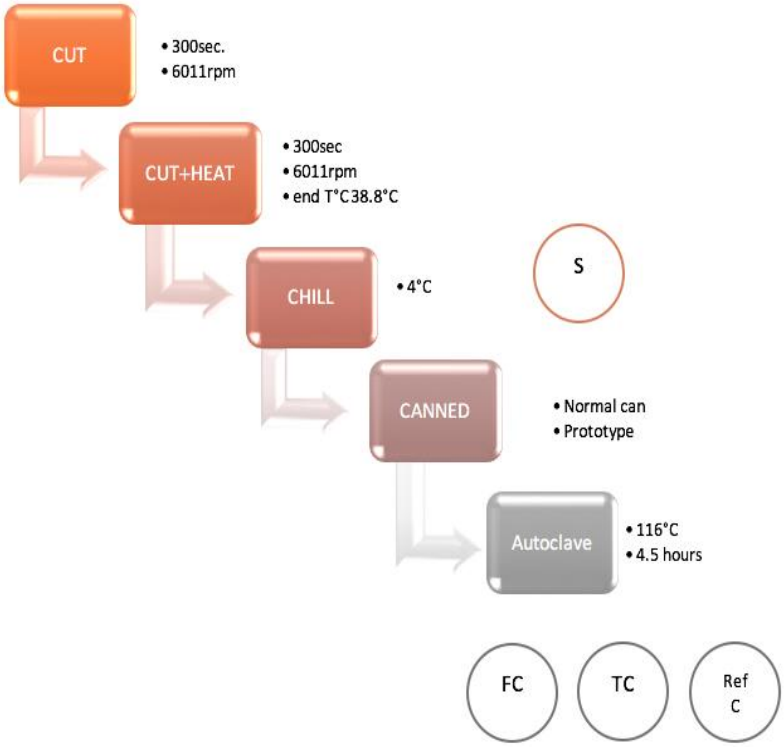

Fig. 1.30: Autoclave samples preparation.

Ref C is a kale purée sample canned in a commercial can. TC is a special toroid can (designed as a hollow cylinder), while FC is a can with the same material of TC but without the cylindrical separator.

Samples FC, TC and Ref C have been treated with autoclave (multi-process horizontal autoclave AR091, jbt FoodTech-UK).

Sample preparation and storage

Part of the samples were analysed fresh (after defrosting), others were used freeze-dried.

Fresh material

Fresh kale and Brussels sprout purées have been stored in the freezer at -40°C. Before the analyses, frozen samples were defrosted in a water bath at 20-30°C until their complete defrosting.

Freeze-dried material

30 g of kale purée from each sample were used to fill a petri dish in a thin layer, to have the lyophilized sample. After that, they were stored in the freezer at -40°C overnight.

Purée samples were freeze-dried before the analyses. The freeze-drying procedure was optimized using VirTis® BenchTop™ "K" Series Freeze Dryer (USA) equipped with a vacuum pump (Edwards RV5 – manual pump - UK), a Stainless Steel Multi Port Drum Manifolds with 12 valves and a Bulk Racks–Unheated with 3-shelf configuration (200 mm) (Fig. 1.31).

Fig. 1.31: Freeze-drying system.

The process g optimization has been performed by testing using different times. At first, samples were processed for 72 hours and successively for 48 hours. Afterward, as both treatment times were found to be undue, in some samples, a shorter drying time of 24 hours was chosen. Thereafter each sample was weighted and the powders were stored in zip lock bags and shielded from the light. These powders were subsequently used for various analyses.

Analysis

Water activity, pH and Dry Matter Determination

Water activity (A_w) was measured using a water activity meter (AQUALAB 4 Series - METER Group). The fresh sample was put into a sample cup until its edge. Successively the filled cup was placed in the machine. Measurements were performed one time. Samples pH were recorded with the pH meter (SevenGo Duo™- Mettler Toledo). Measurements were repeated one time.

For the determination of moisture content, 3 g of each sample was used to fill a disposable sample dish with 3 g of fresh material and place it in the Moisture Analyzer (HC103 – Mettler Toledo GmbH, Switzerland), with an end temperature of 135°C). The temperature increased until a stable weight was reached. Measurements were repeated one time.

Colour measurement

Colour of samples was measured using a UV-Vis spectrophotometer (Sensing Unveils CM-5, Konica Minolta Sensing, Osaka, Japan). A cylindrical glass was filled with samples and the

measurements were performed three times for each sample. Inclusion of air bubbles was avoided. The CIE colour coordinates L*, a* and b* components were recorded.

According to CIE concepts, the human eye has three colour receptors red, green and blue and all colours are combinations of those. CIE L* a* b* colour space (Fig. 1.29.) was devised in 1976 to provide more uniform colour differences in relation to human perception of differences.

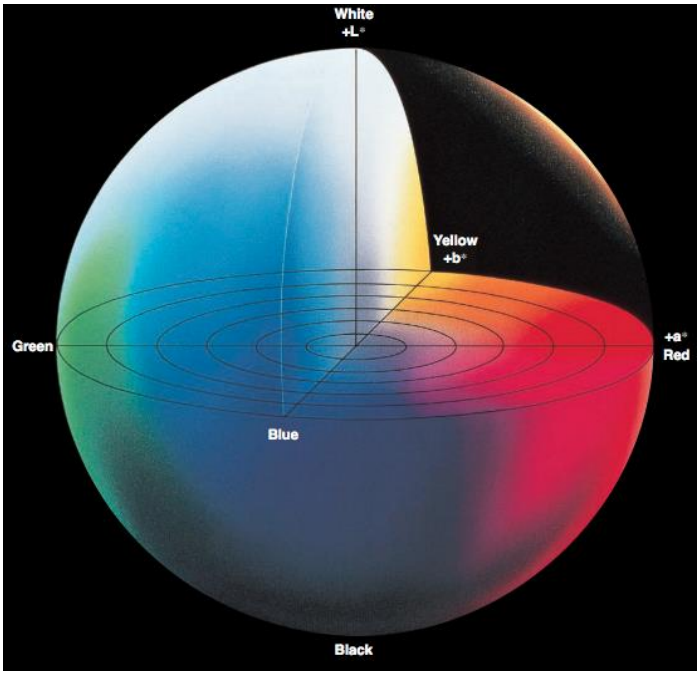

*Fig. 1.32: CIE L*a*b* colour space. L* indicates lightness, from white=100 to black=0; a* and b* are XY colour coordinates indicating colour directions; a* is the red–green axis, b* is the yellow and blue axis; the center is achromatic gray. Hue is the location around the circumference and saturation is distance from center.*

Total colour difference, ΔE, was calculated from L*, a*, and b* values, using Hunter-Scotfield's equation:

$$\Delta E = \left[(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2 \right]^{1/2} \quad \text{Eq. 1}$$

Perceivable colour differences can be classified as not noticeable (0.5-1.5), slightly noticeable (1.5-3.0), well visible (3.0-6.0) and great (>6.0) (Cserlhalmi et al., 2006).

Chlorophyll determination

Total chlorophyll, chlorophyll a and chlorophyll b were determined according to the procedure described by Armesto et al., (2017).

0,26g of sample was homogenized in 10 ml of acetone (80% v/v) using a vortex mixer. The mixture was then centrifuged at 4000xg for 5 min at 4 °C (Sorvall™ LYNX 6000 - Thermo Scientific™, Germany). The residue was re-extracted two times under the same conditions and the resulting supernatants were combined and filtered through a 0.22µm syringe filter

(Millex® Syringe Filters with Hydrophilic Durapore® PVDF Membranes). The total chlorophyll, chlorophyll a and chlorophyll b content were measured at 645 nm and 663 nm using a spectrophotometer (Ultrospec 2100 pro, UV-Vis spectrophotometer, Amersham Bioscience, Uppsala, Sweden) and calculated using the Arnon's equations:

$$Chlorophyll\ a\ = \frac{12{,}7 \times A\ 663 - 2{,}69 \times A\ 645 \times mL\ Acetone}{mg\ purée}$$

$$\text{Chlorophyll b} = \frac{22{,}9 \times A\ 645 - 4{,}68 \times A\ 663 \times mL\ Acetone}{mg\ purée}$$

$$Total\ chlorophyll\ = \frac{20{,}2 \times A\ 645 + 8{,}02\ A\ 663 \times mL\ Acetone}{mg\ purée} \qquad \text{Eq. 2}$$

Where Abs_{645} and Abs_{663} represent the value of absorbance at 645 nm and 663 nm, respectively. Measurements were performed two times for each sample.

Wet sieving analysis

Particle size was determined on purée samples, in two steps. The first one was by wet sieving and it was used to separate larger particles from smaller ones. Measurements were performed one time per sample, by using a Retsch sieving system (AS 200, Retsch, Haan, Germany) equipped with 3 sieves with screen openings of 2 mm, 1mm and 500 µm from top to bottom. Both the top and bottom of the sieving system were adapted for water circulation (Fig. 1.33).

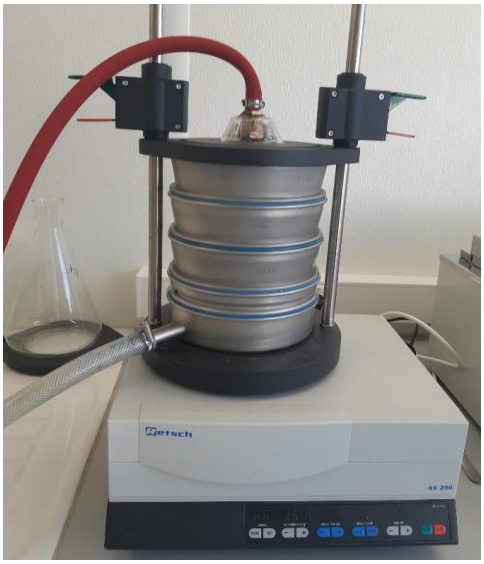

Fig. 1.33: Wet sieving system.

An amplitude of 1,5 was used. First, we measured the water flow 5 times obtaining an average of 1719 mL/min. Approximately 200 g of kale purée was dropped on the first sieve and diluted with 600 g of water to cover the whole surface of the sieve. Sieving was carried out under water circulation for 15 min and without water for 5 min. For each sample liquid and solid moiety was collected separately. The liquid one was gathered in a Duran bottle shown in figure 1.34.

Fig. 1.34: Kale purée liquid moiety.

Each sieve was then carefully rinsed with deionized water to collect particles (solid moiety). The mixture of each sample was filtered through a filter paper (40 ashless, filter paper, Whatman), as in fig. 1.35. Once filters were dried, they were weighted and data were expressed in mass (%).

Fig. 1.35: Kale purée solid moiety.

<u>Particle size analysis</u>

In the second step the particle size of the smaller particles (< 500 µm) were measured using light scattering (Mastersizer; Malvern Instruments Ltd, Malvern, UK) connected to a cell for liquid measurements (Hydro 2000S mixing, Malvern Instruments), shown in fig. 1.36. Samples were analysed by using an absorption of 1 and a refractive index of 1,6.

Fig. 1.36: Mastersizer.

A few droplets of each sample were pipetted into a stirred (200 rpm) and pumped (750 rpm) tank filled with deionized water (1000 ml) until an obscuration of 8% polarization intensity differential scattering was achieved.

The particle size distribution was calculated from the intensity profile of the scattered light using the instrument software (Mastersizer 2000, MIE method). Following magnitudes were determined:

- d(v, 0.1) – the value of particle size below which there is 10% of sample volume;
- d(v, 0.5) – the value of particle size below which there is 50% of sample volume;
- d(v, 0.9) – the value of particle size below which there is 90% of sample volume;
- Span – evaluation of distribution width, calculated as the difference d(v, 0.9) and d(v, 0.1) divided by d(v, 0.5);
- D[3,2] – the area-based mean diameter;
- D[4,3] – the volume-based mean diameter.

Data were expressed in µm. Measurements were performed three times for each sample.

Enzyme activity measurements

Polyphenol oxidase activity

The extraction procedure for Polyphenol oxidase activity (PPO) was adapted from the method described by Liu et al. (2014).

Oxidation of catechol by PPO present in the extract results in the formation of the brown-coloured o-quinone compound. Results are expressed in percentage of the average residual activity in comparison with the reference which has an activity of 100%.

Measurements were performed two times for each sample.

Peroxidase activity

The extraction procedure for peroxidase activity (POD) was adapted from the method described by Yi et al. (2014).

Results are expressed in percentage of the average residual activity in comparison with the reference which has an activity of 100%.

The extraction was carried out in duplicate.

Myrosinase activity

The extraction procedure for myrosinase was adapted from the method described by Oliviero et al. (2014), while for the determination the Van Eylen et al. (2006) article was used.

Glucosinolates analysis

The extraction and the determination procedure for glucosinolates was adapted from the LC-MS method described by Sun Zhang Chen et al. (2016). This newly optimised method is currently under research at ILVO and will at a later time be published in a peer reviewed scientific journal.

Organic acid profile determination

Extraction and quantification procedures were performed according to Wibowo et al. (2015).

Results and discussion

Relationship between water activity and moisture content

All Brussels sprouts samples purée had a high-water activity value, more than 0,98. This confirms that they are susceptible to spoilage (such as softening, yellowing, loss of nutrients) (Kebede et al., 2015; Shi et al., 2016). As a result, the shelf life of Brussels sprouts purée is short and it is affected by the storage temperature, although it could be prolonged with heat treatments.

Since it is known that water activity is related to the moisture content in a non-linear relationship and has an influence on enzyme stability, water activity was measured for Brussels sprouts purée, this relationship is shown graphically in figure 1.37.

This relationship between water activity and moisture content at a given temperature is called the moisture sorption isotherm. These curves are determined experimentally and constitute the fingerprint of a food system.

Isotherms were not built for this research, but they could be of interest to help in the prediction of product stability over time in different storage conditions, and specifically for myrosinase, to find critical relationship points where enzyme stability increases or decreases.

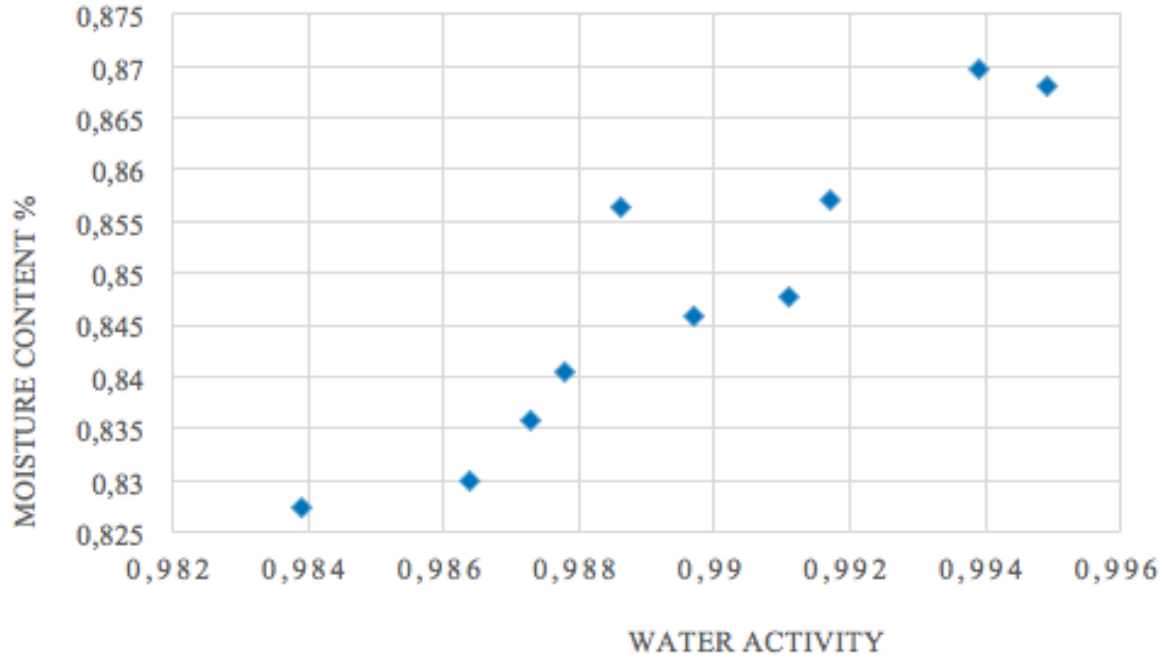

Fig. 1.37: Relation A_W vs. MC% on fresh Brussels sprouts at room temperature (25°C).

Colour and chlorophyll content results

Brussels sprout purée sample

Figure 1.38 shows the chlorophyll content of cooked and blanched samples, respectively.

Chlorophyll content decreased after heat treatment (Figure 1.38 a & b), consistent with the temperature effect on chlorophyll stability and chlorophyll a and chlorophyll b conversion to the respective pheophytins (Steet and Tong, 1996).

Comparing chlorophyll, a and b, figure 1.38 a shows that the latter is less heat-sensitive then chlorophyll a.

As regards the total chlorophyll content (Figure 1.38 b), the cooked sample shows a higher value than the blanched one.

It is clear that the amount of chlorophyll degradation is directly related to the intensity of the thermal treatment, which is characterized by temperature and the duration of heating and holding.

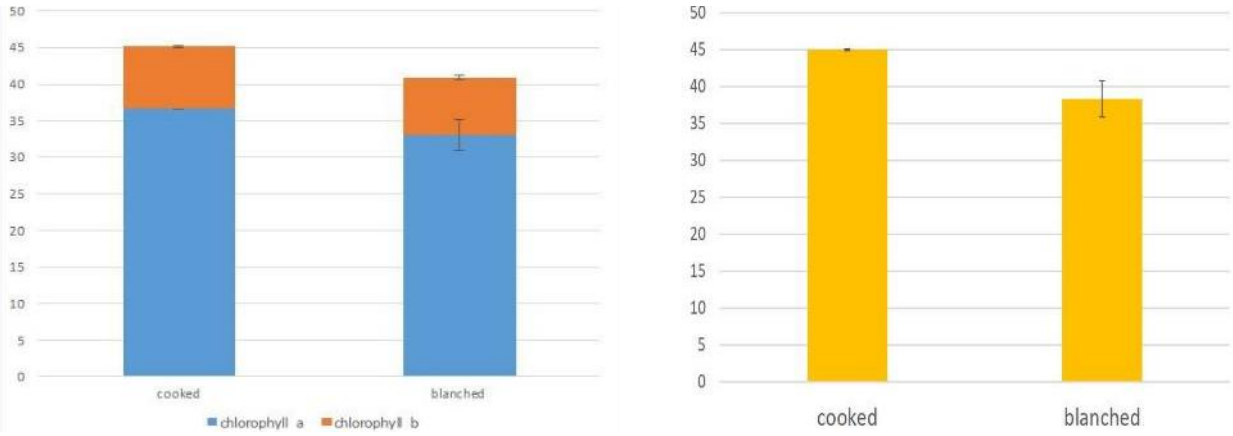

Fig. 1.38: Chlorophyll composition (mg/g) of Brussels sprout purée. Error bars represent the standard error of measurements (n = 2).

Kale purée sample

Figure 1.39 shows the results of colour changing among samples.

F+H(L) and B+F(L) are the only samples that will be taken into account because the others didn't have a clear phase separation (solid or liquid) during the process of production. They have a very similar and visible colour difference (E > 6.0), and both are higher than the reference (F-). Probably, the high value of B+F(L) standard deviations means that there were some problems during this sample preparation.

The colour kale purée detection shows that the loss of green brightness in samples treated has to be accepted, insofar as marketable products are wanted.

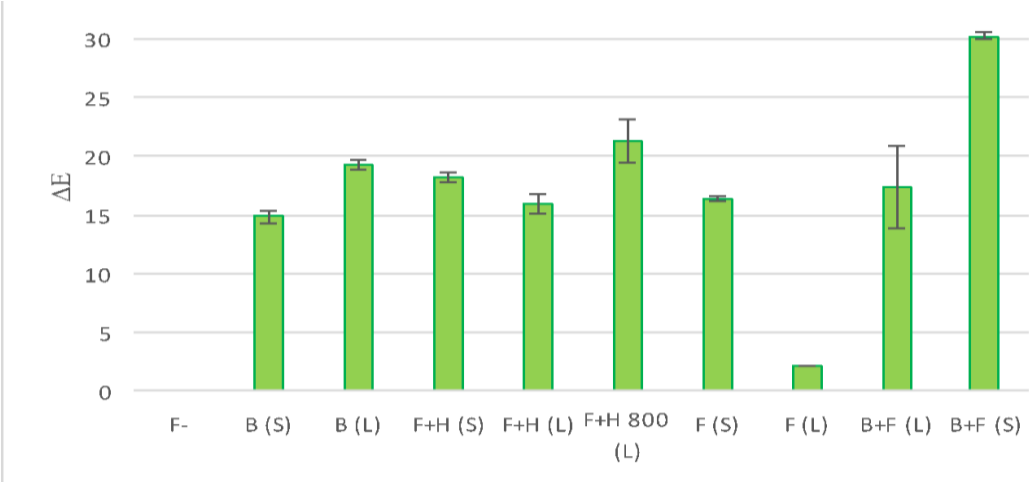

Fig. 1.39: Colour difference of kale purée samples. Error bars represent the standard error of measurements (n = 3).

Table 1.16 confirms what was mentioned above, indeed if the CIE L* a* b* colour space is taken into account, both F+H(L) and B+F(L) are far from the reference but F+H colour coordinates are closer to F^- than B+F(L) ones.

Table 1.16. Colour values and total chlorophyll content of kale purée samples.

	L*	a*	b*	Total chlorophyll (mg/g)
F⁻ (ref.)	18,66±0,38	-14,06±0,33	32,18±0,65	606,50±15,24
F+H	25,77±0,86	-6,51±0,59	44,18±1,29	433±4,16
B+F	27,29±1,14	-1,71±0,15	40,70±4,10	407,11±4,02

As regards chlorophyll, the high intensity thermal treatment (blanching at 95°C) resulted in a significant decrease of the total chlorophyll compared with the reference.

This result is concordant with articles quoted previously, indeed in broccoli juice chlorophyll degradation to pheophytin occurs at temperatures exceeding 60°C (Weemaes et al., 1999). The higher accumulation of pheophytin in broccoli juice occurred at 80°C, decreasing the a* colour coordinate; pheophytin, in turn, decomposes to other degradation products (Weemaes et al., 1999). Heat treatment of Thompson seedless grapes decrease its chlorophyll content (Zheng et al., 2014), changing colour from bright green to olive brown as a consequence of the conversion of chlorophyll (a and b) to their respective pheophytins, and further degradation to pyropheophytins (Steet and Tong, 1996).

Canjura et al. (1991) reported that high-temperature and short-time (blanching) treatments generally provide good results in preserving the green colour immediately after processing. Venning et al. (1989) demonstrated that chlorophyll stabilization can be achieved in frozen foods at temperatures below -18°C or -20°C.

But, even so, results show that the B+F(L) sample (frozen at -20°C between the heat treatments) is the furthest sample from the reference. The reason could be to the temperature and the duration of the heating process, but also to the storage conditions.

Particle size determination

Understanding the influence of processing on microstructure of foodstuff will help to design food products with improved organoleptic parameters such as flavour and texture and also micronutrient bioavailability (Aguilera, 2000). The rheological properties and flow behaviour on purée products are expected to depend on both the soluble solids in the serum phase and the particle volume fraction of insoluble solids. Processing conditions such as shear and thermal treatments could influence particle parameters: size, morphology, hardness, inter-particle forces and the number of particles in the dispersions (Macosko, 1993; Rao and Qiu, 1989).

For these reasons, the effect of processing on particle size distribution (PSD) was evaluated by laser diffraction. To separate larger particles from smaller ones, a first passage of wet sieving was performed for each sample.

Wet sieving results

Kale purée sample, Vaculiq method

After having the filters with the sample allowed to dry 24 hours, they were weighed and the weight of an empty filter paper was subtracted from the former. In this way, the weight that was left on the different sieves was obtained. Taking into account the number of samples that were weighed, the particle size distribution is given as a percentage. Table 1.17 and shows the samples results.

Table 1.17. Wet sieving method results of samples.

B(S)		
Sieve	Weight (g)	%
2mm	0,12	0,06
1mm	0,09	0,04
500µm	1,02	0,51
<500µm	198,80	99,39

F-		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,04	0,02
500µm	1,26	0,63
<500µm	198,73	99,35

F(S)		
Sieve	Weight (g)	%
2mm	0,13	0,08
1mm	0,25	0,15
500µm	1,39	0,85
<500µm	162,48	98,92

F+H(L)		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,00	0,00
500µm	0,15	0,07
<500µm	199,86	99,93

B+F (S)		
Sieve	Weight (g)	%
2mm	0,01	0,00
1mm	2,75	1,37
500µm	40,98	20,49
<500µm	156,28	78,13

F(L)		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,00	0,00
500µm	0,22	0,11
<500µm	199,78	99,89

F+H 800 (L)		
Sieve	Weight (g)	%
2mm	0	0
1mm	0	0
500µm	0,02	0,01
<500µm	180,01	99,99

B(L)		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,00	0,00
500µm	0,36	0,18
<500µm	199,70	99,82

F+H(S)		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,05	0,03
500µm	4,09	2,27
<500µm	175,89	97,70

B+F (L)		
Sieve	Weight (g)	%
2mm	0,00	0,00
1mm	0,02	0,01
500µm	0,73	0,36
<500µm	199,65	99,81

From the results of sample F- it becomes clear that a very small amount of purée sample is left on the different sieves. From this, we can conclude that a large part of the particles present in the sample has a size smaller than 500 µm. The B+F(S) sample has significantly much more weight <500 µm sieve than the other samples. This may be because the filters with these samples were still not completely dry after 24 hours. This sample filter has been dried further and weighed after 48 hours. It was noticed that also at this time the filters were not completely dry due to a large number of particles present on them. It has caused an error on the weight

for the excess of water in each filter. What became visually clear is that there were more particles on the 500 µm sieve of sample B+F(S) than on those of the other samples. The F+H(S) sample also has more particles <500 µm on the sieve compared to the other filters. Nevertheless, the difference between this sample and the other samples is a lot less than the difference between steel B+F(S) and the other samples.

Kale purée sample, autoclave method

Table 1.18. Wet sieving method results of samples.

S		
Sieve	Weight (g)	%
2mm	0,04	0,04
1mm	0,13	0,13
500µm	1,05	1,05
<500µm	98,78	98,78

Ref C		
Sieve	Weight (g)	%
2mm	0,1	0,05
1mm	0,54	0,27
500µm	2,51	1,25
<500µm	196,91	98,43

TC		
Sieve	Weight (g)	%
2mm	0,01	0,00
1mm	0,21	0,10
500µm	1,87	0,93
<500µm	197,93	98,96

FC		
Sieve	Weight (g)	%
2mm	0,1	0,08
1mm	0,30	0,23
500µm	1,72	1,31
<500µm	129,45	98,39

Results show that the majority of the particles of the sample had a diameter less than 500 µm. The difference between the autoclave treated samples are small and the number of particles <500 µm was always between 98% and 99%.

Mastersizer results

VaculIQ®: kale purée sample

Table 1.19 shows the average of the results.

The F^- sample was used as the reference. If the value d (0.1) is considered, it is noticeable that for most of the samples it is lower than the same one of the reference, but F(L) sample is an exception. Moreover, the d (0.5) value of most of the samples is lower than the same one of the reference, sample F(L) is the exception also in this case. As regards the d (0.9), the F(L) and B+F(L) samples have higher values than the d (0.9) value of the reference.

Results show that most of the particles samples have a smaller diameter than the particles in the F^- sample. F+H(L) samples are always the lowest values among samples. For this reason, it's possible that the processing steps that it has undergone, have a major effect on the diameter of its particles, so the diameter of its particles results the smallest. Sample F(L) has values that are higher than the ones of the reference.

Table 1.19. kale vacuIIQ® results.

	D [4, 3]	D [3, 2]	d (0.1)	d (0.5)	d (0.9)
F⁻ (Ref)	298,78±17,59	104,36±14,83	59,67±10,66	270,21±19,87	577,01±19,90
B(S)	271,50±11,08	61,17±3,05	35,78±1,52	229,73±11,11	576,41±20,69
B(L)	230,35±3,40	55,86±0,07	31,72±0,34	184,05±3,88	504,90±5,576
F+H (S)	247,50±5,33	65,74±2,59	30,44±1,163	206,11±4,71	531,84±10,76
F+H (L)	204,58±2,79	54,72±2,22	27,42±1,06	163,66±3,93	449,29±3,24
F+H 800 (L)	230,86±2,95	62,46±4,05	29,37±1,72	195,96±4,71	493,41±6,61
F(L)	326,73±67,27	131,75±44,57	86,48±33,62	300,76±4,71	601,63±6,61
B+F (L)	236,62±3,14	53,31±5,27	29,26±1,46	182,50±2,36	532,87±9,08
B+F (S)	293,32±11,71	81,79±3,07	40,92±1,71	246,76±14,09	623,69±18,62

Differences observed in the particle sizes of F- and F(L) samples would be mainly ascribed to two reasons. The first could be due to a greater or lesser degree of homogenization of the F(L) purée. The second reason could regard heat treatment. It is thus capable of inducing the separation of cell wall polymers as well as the middle lamella which could lead to higher mechanical disruption to bring about particles ranging from particles to cell clusters.

Autoclave: kale purée samples

The average of the results is shown in Table 1.20.

Table 1.20. Kale autoclave results.

	D [4, 3]	D [3, 2]	d (0.1)	d (0.5)	d (0.9)
Ref C	220,04±4,25	63,93±1,28	30,38±0,59	160,90±6,06	503,93±5,87
S	284,69±12,89	85,31±5,71	39,90±2,61	245,24±15,39	597,31±19,19
TC	219,56±1,89	64,67±0,51	31,05±0,39	157,12±2,10	506,43±3,32
FC	244,17±3,30	66,92±1,82	30,86±0,86	182,05±3,51	557,39±5,86

For the kale samples that were processed in the autoclave, the results of each parameter are very similar among samples. The d (0.5) value of the different samples is between 157 µm and 254 µm. S sample (reference) always shows the largest D value, which indicates that with the increasing time of mechanical treatment values of parameters that characterise diameters of particles decrease. This result was also reported during different temperature treatment of tomato concentrate (Probola et al., 2015).

Enzyme analysis

Polyphenol oxidase and Peroxidase activity results

Enzymatic browning of leafy vegetables is considered one of the most important defects because it is easily noticeable. Browning reactions are due to the PPO enzyme, although some attribute at least a partial role of POD.

Results of these analyses showed that untreated samples had a high activity of PPO and POD. Heath treatments cause a decrease of these activities with some being more efficient than others. These results are in accordance of what is found in the literature.

A more detailed discussion of the results will be published in a peer-reviewed journal.

Myrosinase activity results

Myrosinase is found in all glucosinolate-containing plants, especially in Cruciferae. Myrosinase catalyzes the hydrolysis of glucosinolates, a group of sulfur-containing pseudo glycosides, and it is responsible for hydrolyzing glucosinolates upon plant tissue disruption. This hydrolysis results in the formation of sulfate, D-glucose, and a series of sulfur- or nitrogen-containing compounds such as isothiocyanates, thiocyanates, nitriles, and thiones. These compounds are responsible for the specific flavour and aroma of several cruciferous vegetables (Gatfield and Sand, 1983; Wilkinson et al., 1984).

Ludikhuyze et al. (1999) studied the thermal inactivation of myrosinase from lyophilized broccoli, at temperatures ranging from 30 °C to 60 °C. The activity remained rather constant at 30 °C, and significant inactivation occurred at 40 °C and higher. A treatment of 3 min at 60 °C was sufficient to reduce enzyme activity by 90%. These results show myrosinase from broccoli to be rather thermolabile.

Dunford and Temelli (1996) reported inactivation of myrosinase in crude rapeseed extract to proceed at temperatures >65 °C, whereas inactivation in flaked seeds required temperatures in the range of 90-100 °C.

Wathelet et al. (1996) investigated the inactivation of myrosinase from Brussels sprouts by blanching. The study shows that treatments of 10 min at 90 °C, 5 min at 95 °C, and <5 min at 105 °C (steam blanching) resulted in >90% activity loss. Glucosinolate concentrations are reduced by 13 or 3% respectively by these treatments. While the reduction in glucosinolates is mostly a result of leaching after water blanching Brussels sprouts, the decrease in plant myrosinase activity is positively related to blanching temperature.

For both red and white cabbages, myrosinase is inactivated >90% after a treatment at 70 °C for 30 min (Yen and Wei, 1993). The higher temperatures reported for myrosinase inactivation in these cases may be due to limitations in heat transfer involved in the heat treatment of whole vegetables as compared to vegetable enzyme extracts.

Method optimization

To determinate the myrosinase activity, two different start samples (fresh material and freeze-dried material) and two different size filters were used.

Filters and material comparison

To determine which filter (Amicon Ultra-15 or Ultra-4 cut-off 30kDa, Millipore) gives the best result, both of them were tested on fresh material samples and freeze-dried material samples. The results are shown in Table 1.21 and 1.22.

Table 1.21. Fresh purée results.

	Ultra-15 30kDa, Millipore				**Ultra-4 30kDa, Millipore**			
	4.1	4.2	6.1	6.2	4.1	4.2	6.1	6.2
Units myrosinase / g dry matter	0,3546	0,3133	2,7218	2,7487	0,5705	0,0455	1,8192	2,5103
Δ	0,0413		0,0269		0,525		0,6911	

Table1.22. Freeze-dried sample.

	Ultra-15 30kDa, Millipore				**Ultra-4 30kDa, Millipore**			
	4.1	4.2	6.1	6.2	4.1	4.2	6.1	6.2
Units myrosinase/ g dry matter	0,0108	0,0799	0,2384	0,2122	0,0294	0,0010	0,0852	0,0878
Δ	0,0691		0,0262		0,0284		0,0026	

Comparing the results, it was clear that the ones of fresh material were much more similar among the repetition of the same sample, and also that the big filter had a major extraction efficiency.

Because of this, it was decided to use the fresh material and the big filter, a combo that results demonstrate to be the most effective.

Vaculiq Brussels sprout

Table1.23 shows the results of the raw sample (6) and the cooked in water (4) one to compare with each other.

Table 1.23. Brussels sprout results.

	4.1	**4.2**	**6.1**	**6.2**
Units myrosinase/ g dry matter	0,0108	0,0799	0,2384	0,2122
Mean	0,0454		0,2253	

Sample 4 underwent a cooking process in water for 15 minutes at temperatures between 96 °C and 100 °C. The 6 sample was not processed but directly stored in the freezer at -40°C. The results show that the activity of the myrosinase enzyme in the raw sample (6) is significantly higher than the activity of the myrosinase enzyme in the processed sample (4). This result is consistent with those reported in the literature quotes above, in which a heat treatment >70°C can inactivate this enzyme. As reported by Wathelet et al. (1996), the decrease of myrosinase activity is positively related to blanching temperature.

Since the myrosinase enzyme is necessary to promote the conversion of glucosinolates into health-promoting components, the results demonstrated that in the raw sample more glucosinolates will be converted to these components.

Autoclave kale sample

Table 1.24 shows the myrosinase activity in kale purée samples prepared with different heat treatment and processed with the autoclave.

Table 1.24. Kale results

	S1	S2	RefC1	RefC2	TC1	TC2	FC1	FC2
Units myrosinase/ g dry matter	0,9449	1,0825	1,0605	1,1391	0,1014	0,2952	1,4908	0,9872
Mean	1,0137		1,0998		0,1983		1,2390	

Results clearly show that the FC sample has the highest enzyme activity. This sample was put in a can of the same material of the toroid can but without the hollow. This result is in strong contrast with the previous ones, because the S sample (reference, not treated) has the lowest myrosinase activity. As suggested by Ludikhuyze et al., (1999) the application of low pressure (<350 MPa) resulted in retardation of thermal inactivation which indicates an antagonistic or protective effect of low pressure. The samples TC1 and TC2 however have extremely low myrosinase activity compared to other samples. These samples were heated up much faster than the others and were therefore much more subjected to the inhibitory effects of the heat treatment.

Glucosinolates results

Samples 5 and 6 are the raw samples and are taken as a reference. It is striking that no glucosinolates were detected in these samples. Glucosinolates were also not detected in the samples that were blanched with steam. An explanation for this could be that the myrosinase enzyme in these samples was not inactivated, thus it degraded the glucosinolates present in these samples into its breakdown products.

A few glucosinolates were always detected in all the other samples. This indicates that in these samples the myrosinase enzyme was probably partially or completely inactivated so that not

all glucosinolates were degraded and could be detected. Another striking feature is that glucoraphanin was not detected in any sample. Probably this glucosinolate is not present in Brussels sprouts.

These results are consistent with those reported in the literature quotes above. Moreover, as Cartea and Velasco (2008) reported, the glucosinolates with the highest concentration detected were: progoitrin, synigrin, gluconapin, and glucoraphanin.

Organic acids results

Citric, oxalic and malic acids were the major organic acids found in sprouts of all the varieties accounting for 67%, 19% and 13% of the total organic acids, respectively. Citric and malic acids are known for being present in large amounts in all plant materials (seeds and leaves) (Fernandes, 2011) as they accumulated in plant tissues (Harborne et al., 1999). The presence of oxalic acid in green vegetables such as Brussels sprout, broccoli, and kale is well-recorded (Judprasong et al., 2006; Armesto et al., 2018).

Figure 27 shows the results of the organic acids detected in this research. Malic acid was found in the raw and then blanched by steam samples. Probably the heating treatment performed in water reaches temperatures that caused the degradation of this organic acid. The lactic acid was detected in all the treated samples, but not in the raw samples. The citric acid was found in all the samples with a similar concentration. Lastly, the oxalic acid was in the raw and treated by steam samples but was not found in the samples treated in water. These results show that samples blanched and cooked in water are the poorest in organic acid. Resulting in a decreasing of the organoleptic quality of the product.

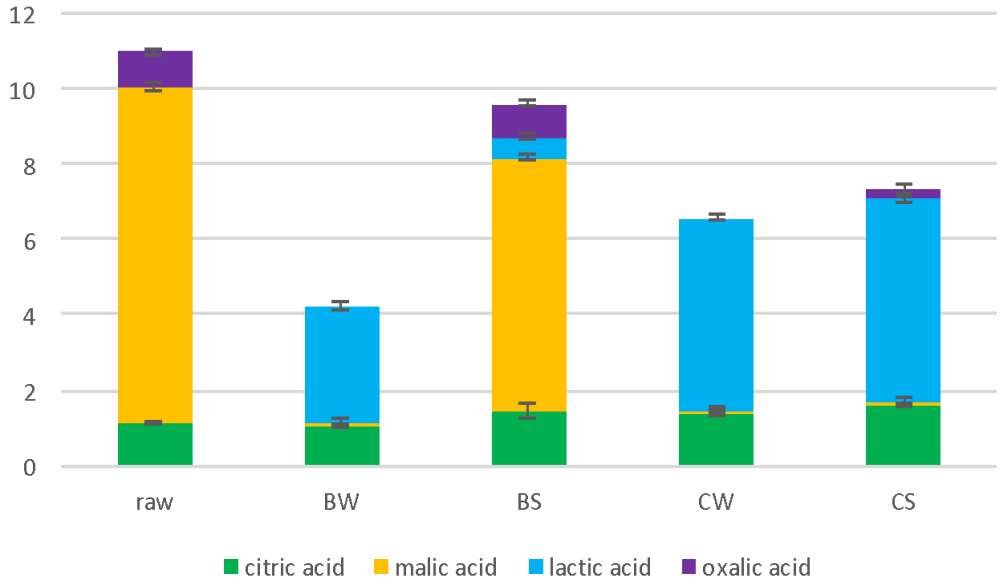

Fig. 1.40: Organic acid composition of Brussels sprout purée. Raw sample = 5 and 6; blanched in water (BW) = 1 and 2; blanched in steam (BS) = 9 and 10; cooked in water (CW) = 3 and 4; cooked in steam (CS) = 7 and 8. Error bars represent the standard error of measurements (n = 2).

Conclusions

Different heat treatments on kale and Brussels sprout purée were investigated on a pilot-scale to obtain a marketable product that maximally preserves the organoleptic and nutritional quality of purée. The study involved a targeted approach which explores the overall process of the kale and Brussels sprout purée. It was performed by studying the chemical and physical characteristics of the products which are linked to the product's appearance and texture.

Kale purées were processed with VaculIQ® or in the autoclave. The former can be distinguished from each other, by the cooking treatment. B samples were blanched in steam while F samples were heated at 20°C or 60°C. The latter were divided into different types of can (normal, toroid, toroid without the hollow) and treated in the autoclave to reach 116°C.

The amount of chlorophyll degradation is directly related to the intensity of the thermal treatment, which is characterized by temperature and the duration of heating and holding. This phenomenon is directly correlated to the loss of the green colour which moves to olive brown pheophytin and pyropheophytin pigments in the blanched kale and Brussels sprout treated with VaculIQ®, while for the autoclave treated samples in the TC sample.

Differences in consistency were observed for the kale F(L) sample, but they were not significant in the autoclave samples. It could be caused by different degrees of the product's homogenization before the analysis.

Polyphenol oxidase and peroxidase activity measured in the samples were consistent which is found in the literature, that these are inactivated at temperatures over 100°C.

Myrosinase in Brussels sprout samples was totally inactivated, while results of the autoclave samples suggested that pressure could be a protection factor of this enzyme.

With regard to glucosinolates, those with the highest concentration were: progoitrin, synigrin, gluconapin, and glucoraphanin. They were detected in all the Brussels sprout samples, except for the raw material.

The detection of the organic acid profile of Brussels sprout showed that the blanched and cooked in water samples lost less than others.

In general, the blanched in water and the autoclave treatment reduced most favourable qualities of kale and Brussels sprout purée. An intermediate thermal process could be the best choice to preserve the nutritional and organoleptic quality. This could result in a more stable and more marketable product under refrigerated conditions.

1.4. General discussion, recommendations and conclusions

In this chapter on pre-processing of various by-products from the agriculture and horticulture, we found that there exists an amplitude of opportunities to valorise unused (or underused) by-products and pre-process them accordingly to open new pathways towards the development of high-value processed foods. This concept of utilising by-products and food waste fractions in a more effective and sustainable way and to literally turn trash into treasure strongly adheres to the rising concerns that the general public has regarding the high amounts of unused waste streams that the food industry is generating. Many of these wastes are in fact perfectly suited to be processed into high quality value-added products for human consumption, yet are often not considered for reasons that vary from convenience (e.g. investing in an additional effort during the production or extra processing and handling steps) or the lack of an established market to economically profit from these source materials.

Additionally, many processed products that incorporate otherwise unused by-products also tend to have a strong health promoting potential, thus appealing to an ever-growing crowd of supporters and niche product enthusiasts that seek out healthy, sustainable and eco-friendly foods.

The most important aspect of the pre-processing of by-products is to initially investigate the potential of these products in terms of compounds (and their concentrations) that may have an impact on human health. This new knowledge could result in unique selling points for these newly developed ingredients. It is indeed very important to know the product well before developing specific applications for them. After all, not all plant parts may contain equal amounts of compounds such as polyphenols or fibres. Using by-products from mainstream crops opens the door for a broad spectrum of applications, depending on what part of the produce is being valorised.

On the other hand, the pre-processing of by-products by extraction of valuable compounds such as dietary fibres may generate on its turn new by-product streams that may be discarded or left unused. In the spirit of a circular economy where all possible fractions and by-products are valorised on their turn to maximise the efficiency of the overall production and processing chain of the product. In any case, reducing the amount of un(der)used waste streams greatly improves the sustainability of the production chain and allows to create more profit for the growers and processors.

2. Value-added processing techniques

2.1. Introduction

This chapter investigates the practice of value adding in food production by applying innovative processes and developing novel applications (value-added products) for human food applications, starting with unused or currently discarded by-products and food waste fractions from small farming producers and seeking short chain processors who can create healthy processed foods that are both healthy and have a great appeal to the general public. Processed foods are "value-added" products, referring to the fact that the source ingredients, in our case by-products, are transformed into a processed product of higher value that can be commercialised. Additionally, we aim for end-products with added health benefits.

For the development of value-added products both local initiatives and trends in food consumption were taken into account along with the focus on healthy, sustainable and natural foods. The emphasis was on the production of minimally processed foods to minimise the alterations to health benefits provided by compounds that are naturally present in the source material, and on convenience foods that are easily accessible for both a large public and specific target audiences such as vegans and eco-minded food enthusiasts.

ILVO strongly focuses on the use of innovative techniques to improve product quality and maximise the preservation of bioactive compounds in processed food. A unique technology that can easily be adapted to various kinds of source materials is the VaculIQ® vacuum press, now under license by GEA[1], and presents various advantages for the oxidation free processing of juices. VaculIQ® is capable of pressing virtually any kind of fruit or vegetable, typically with a cutting or dicing step prior to the pressing, to obtain fresh additive free juices with a maximum of unaltered health promoting compounds and a press cake that is rich in fibres and other remaining compounds that can be in turn used for further processing to extract for example dietary fibres.

The application of such innovative technologies is currently not mainstream but could bring huge benefits to processors who are now working under sub-optimal conditions with standard machinery that is available to them. The introduction of a new generation of processing equipment to the processing industry via novel initiatives such as pilot testing plants and technology hubs such as the ILVO Food Pilot in Melle, Belgium [2] could strongly stimulate the production of improved processed food products and the development of value added products for local produce and open up new market opportunities for a wide range of unused by-products from agriculture and horticulture in Europe.

[1] https://www.gea.com/en/news/trade-press/2019/2019-10-23-GEA-vaculiq-vacuum-spiral-filter.jsp

[2] http://www.foodpilot.be/en/

2.2. Aim

The aim of this chapter is to explore the value-added processing for a selection of by-products and food waste fractions to obtain healthy processed foods that can be valorised commercially in a short chain context.

2.3. Case studies

2.3.1. Tomato juices from de-greened tomatoes

Developed at ILVO

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ILVO
Flanders research institute for agriculture, fisheries and food

Introduction

ILVO, in association with Tomabel (http://www.tomabel.be/), investigated the possibility to process fresh tomatoes (class II) which were unsellable to the fresh market and transform them into market relevant processed food products.

Tomabel was founded in 1996 as a quality label for tomatoes and since then grew into a familiar sounding name for high-quality fruit and vegetables. The Tomabel quality label was introduced in response to the large quantity of bulk products that were then put on the market.

The fresh market criteria used by the Belgian auctions for class II tomatoes are the following:

For regularly formed separate tomatoes:

- Must have a good consumption value
- Sufficient firm flesh and free from dark and very soft spots
- Free from fresh cracks
- Free from significant deformations
- Free from fruits seriously affected by botrytis spot
- If the quality, shelf life and presentation are not adversely affected

Allowed:

- Slight deviations in form and / or development
- Slight deviations in colour
- A less homogeneous colour per packaging unit and per offered lot
- Shrinkage and swelling cracks if not visibly corked
- Sanding damage and cork damage, provided that the surface area is limited to 1 cm^2
- Healed (star) cracks with a total length of at most 3 cm
- Navel formation with curing up to 2 cm^2
- Very narrow elongated flower scar (similar to a seam)
- Slightly dried crowns

Not allowed: strongly deformed tomatoes.

For the development of a value-added product based on class II tomatoes, three recipes for tomato juice were developed in close collaboration with Tomabel. All juices were obtained using the VaquIIQ® vacuum press at ILVO.

Fig. 2.1: Class II tomatoes with visible signs of exterior damage or deformities.

Methods

A tomato juice was obtained after low oxygen pressing of the class II tomatoes with the VaculIQ® vacuum filter press. This juice was pasteurised to prevent spoilage and served as a base ingredient for the three tomato juice recipes that were developed.

The three recipes that were developed are here designated as a tomato-basil juice, a tomato-celery juice, and a spicy tomato juice (Fig. 2.3).

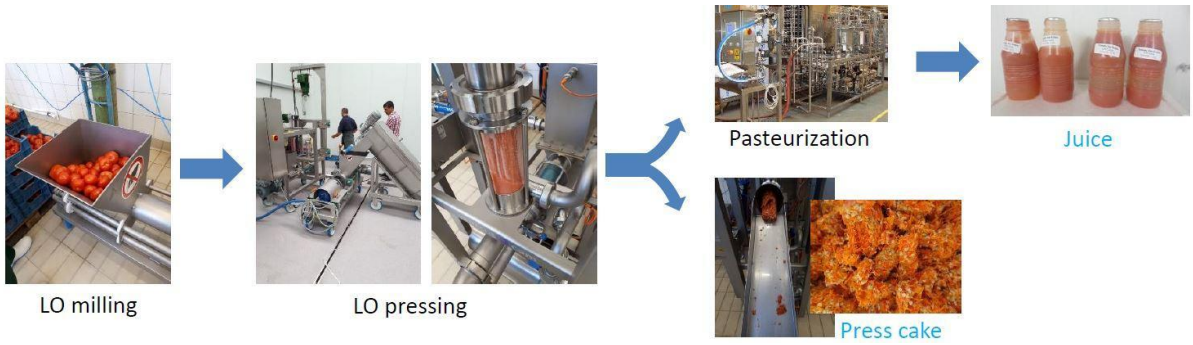

Fig. 2.2: Schematic overview of the processing steps for the fractionation under vacuum (low oxygen pressing) of the tomatoes into a press-cake fraction and a juice.

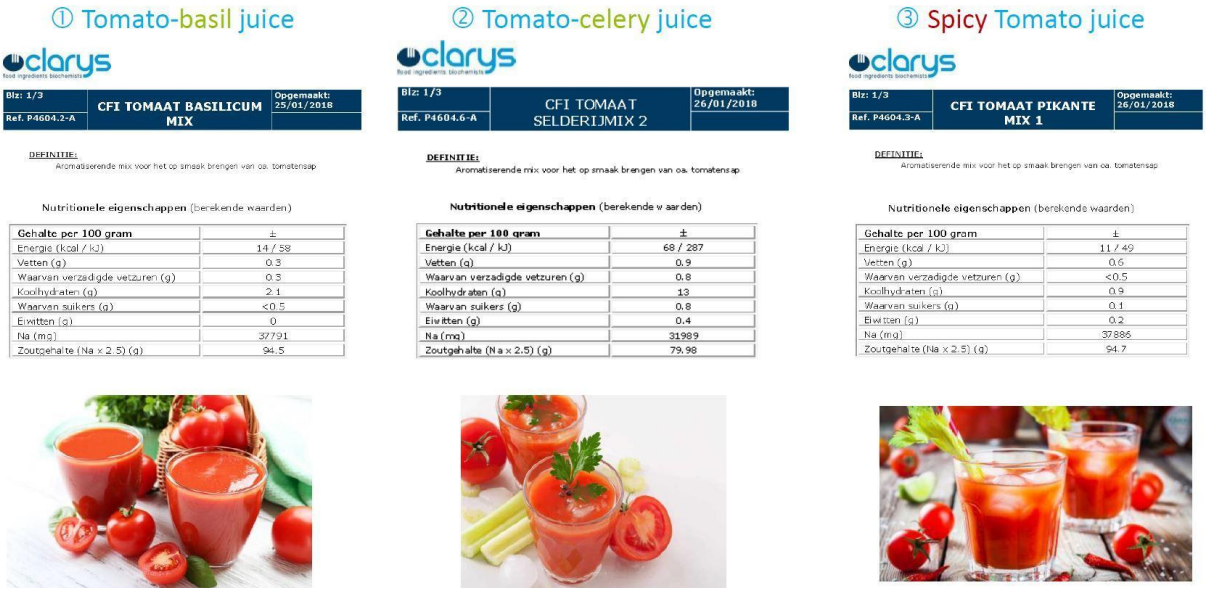

Fig. 2.3: Overview of the three recipes that were developed for tomato juices: tomato-basil juice, tomato-celery juice and a spicy tomato juice.

Results

The tomato juices (3 different recipes, the exact composition of the juices is confidential) that were obtained from the class II tomatoes were subjected to a taste panel to evaluate appreciation with a broad audience and to detect preferences for one of the recipes by the taste panel. The taste panel was composed of a naive group without previous tasting experience to reflect at the best the preferences of the general public (see appendix 1). The tasting was organised at ILVO during its yearly open door's day event on 6 October 2019 at the Technology and Food research unit site in Melle, Belgium, during which visitors could participate in food-related activities, and had the opportunity to taste the tomato juices. This resulted in 384 individual testers that tasted the tomato juices and filled in the taste panel form.

Visitors also received background information on the juices and how they were made, revealing a genuine interest and appreciation of people for food products that are based on food by-products such as the discarded class II tomatoes.

Results of the tasting panel

The tasting panel comprising 384 naive testers revealed that the tomato-basil juice was the most appreciated by the general public. The 'sizes by ranks' table of the results, backed by a Friedman test with a p-value < 0,0001 showed that the tomato-celery juice came in second place and the spicy tomato juice in the third place.

A difference test at the 5% level between the different tomato juices revealed that there was a clear differentiation in preference between the tomato-basil juice and the other two juices.

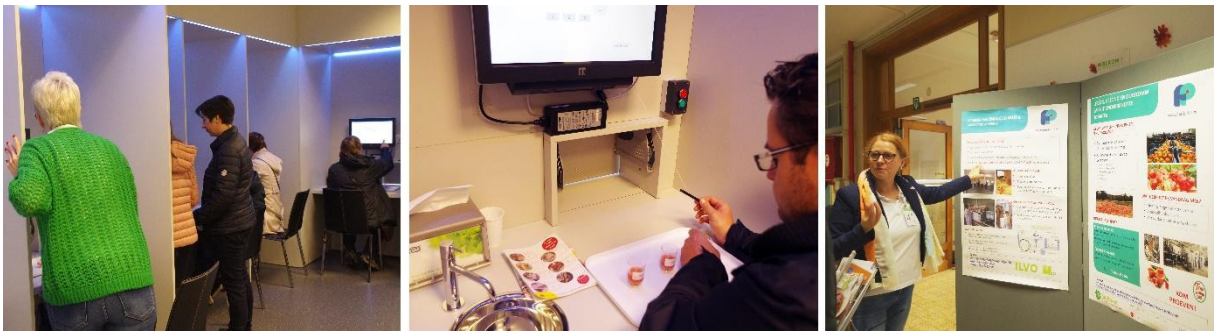

Fig. 2.4: Visitor at ILVO enjoying the participation in a taste panel for tomato juices developed with class II tomatoes.

Table 2.1: Comparison test, the difference between levels with same letter is not significant. Difference limit at 5%

Level	S. Ranks	Groups
Spicy	855	A
Tomato-Celery	797	A
Tomato-Basil	652	B

2.3.2. Product development using residual cooking fluids of legume processing

Developed at ILVO

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Introduction

In the pre-processing chapter of this report we previously investigated the food safety of by-products (cooking fluid) from a company in Flanders (De Hobbit: https://www.hobbit.be/nl), which produces biological meat substitutes. We especially focused on the amount of saponins contained in this liquid, which are very soluble in water and abundantly present in both soybeans and chickpeas. Although these glycoside class molecules (saponins) could be important factors possibly involved in exhibiting the 'whipping' effect of cooking fluid, they are also mentioned to possibly have toxic effects in high concentrations. Therefore, we investigated the concentrations of the total amount of saponins, and compared it with saponins present in other kinds of cooking fluid and the edible legumes itself. The saponin concentrations in industrially produced cooking water from legumes are comparable with those of kitchen-made cooking fluid, and most importantly, are significantly lower than the saponin concentrations in cooked (and edible) chickpeas and soybeans used in many recipes. In short, we can conclude these cooking fluids are thus safe to eat and can readily be used in food preparations and recipes.

Aims

The next goal is to look at how we could use these by-products to eventually introduce them on the market. We were therefore inspired by the 'aquafaba' recipes abundantly found on the internet, in which they use moisture from chickpea food cans as an egg white replacement for vegans. First, we will investigate the actual properties of this egg white replacement a little deeper, and compare the whipping effect, stability, firmness and hardness with these of actual whipped egg white. If these results are beneficial, we will continue this research in the kitchen and start to produce multiple vegan recipes.

Methods

First step: Investigate and compare whipping, foaming capacity, stability, firmness, airiness, hardness and measuring the protein concentration:

Several sources of cooking fluid (chickpea, soybean) were whipped for short periods of 2-5 min, and these whipped foams were compared to the whipped foam derived from egg white, which will be used as a reference. Hereafter we describe the foaming ability (FA%) by measuring differences between the volume of the whipped foam in a cup (Vf), and the volume of the pure fluid before whipping (Vi), and then express the percentage differences in between

(FA (%) = (Vf-Vi)/Vi x 100). These foams were left for 60 min at room temperature and after one hour checked to see if the structure had changed or not, especially whether or not some moisture fluid appeared. The percentage differences of the foam after 60 min was measured, and the volume stability (VS (%) = (100/Vf) x Vs) (Vs=foam volume after 60min) was calculated. We also describe the firmness and airiness visually.

A hardness measurement was done using a Texture Analyzer (Lloyd instruments). Four replicates were used for whipped egg white, and each time 6 replicates were used for whipped chickpea fluid derived from food cans and 6 for kitchen made cooking fluid. The hardness is expressed in Newton (N). For statistics, an ordinary one-way ANOVA is applied, and multiple comparisons are calculated using a Dunnett's test in which whipped egg white is considered as a standard. For whipped soy beans, only one sample is compared to whipped egg white and an unpaired parametric t-test is used.

To measure the proteins present in the 'Aquafaba', it was necessary to first freeze dry the cooking fluid or food can liquid, as the protein content (actually the nitrogen content that serves as a proxy to determine the protein content) was too low to accurately determine the amount of proteins. On the freeze-dried powder, we used the Kjeldahl-method to detect the amount of organic nitrogen present, and thereafter we multiplied the result by 6,25 to calculate the approximate quantity of proteins.

Second step: recipes

If the previous results of whipped cooking fluid appeared to be solid enough for application in recipes as a substitute for whipped egg white, then we proceeded to develop adapted recipes for them. We created recipes for chocolate mousses, vegan butter and a cheese preparation. From this point on the usable cooking fluid will be referred to as 'aquafaba'.

Recipes Chocolate mousse:

Recipe 1: pure chocolate (70%), aquafaba (chickpea food-can), agave syrup.

Melt the chocolate au bain-marie. In the meantime, whip the aquafaba in a kitchen robot on the highest setting for at least 10 minutes, or until the foam has completely reached the stage of stiff peaks. Slowly add the agave syrup while whipping. After the melted chocolate has cooled slightly, add it to the kitchen robot. Continue mixing on the highest setting for about 10 minutes. Spoon into portions and place in the fridge overnight.

Recipe 2: pure chocolate (70%), aquafaba (chickpea food-can), agave syrup, cider vinegar.

Melt the chocolate au bain-marie. In the meantime, whip the aquafaba in a kitchen robot on the highest setting for at least 10 minutes, or until the foam has completely reached the stage of stiff peaks. Slowly add the agave syrup and apple cider vinegar while whipping. Carefully fold the aquafaba foam into the melted chocolate. Spoon into portions and place in the fridge overnight.

Recipe 3: pure chocolate (70%), aquafaba (chickpea food-can), coconut milk (butter), granulated sugar.

Break the dark chocolate into pieces in a bowl and set aside. Heat the coconut cream in a pan until it starts to simmer. Add it to the bowl with the chocolate and stir until the chocolate has melted. In the meantime, whip the aquafaba in a kitchen robot on the highest setting for at least 10 minutes, or until the foam has completely reached the stage of stiff peaks. Slowly add the sugar while whipping. Carefully fold the aquafaba foam into the chocolate-coconut mixture. Spoon into portions and place in the fridge overnight.

Recipes cheese preparation

Recipe 1: Cheese preparation containing cashew nuts: cashew nuts, aquafaba (soybean cooking fluid), apple cider vinegar, vegetable stock, tapioca, nutritional yeast, salt, garlic powder

Add all the ingredients in a blender or kitchen robot (like Thermomix). Blend until the mixture is smooth and contains no lumps. Move the mixture to a pan and start to heat on a low setting. Let the mixture simmer until it has thickened and starts to develop a “stretchy” consistency (comparable to melted cheese). Pour the mixture to a mould, let cool slightly before storing it in a fridge overnight.

Recipe 2: Cheese preparation containing chickpeas: cooked chickpeas, aquafaba (soybean cooking fluid), apple cider vinegar, vegetable stock, tapioca, nutritional yeast, salt, garlic powder

Add all the ingredients in a blender or kitchen robot (e.g. Thermomix). Blend until the mixture is smooth and contains no lumps. Transfer the mixture to a pan and start to heat on a low setting. Let the mixture simmer until it has thickened and starts to develop a “stretchy” consistency (comparable to melted cheese). Pour the mixture into a mould, let it cool slightly before storing it in a fridge overnight.

Recipe 3: Cheese preparation containing soy beans: cooked soybeans, aquafaba (soybean cooking fluid), apple cider vinegar, vegetable stock, tapioca, agar, nutritional yeast, salt, garlic powder

Add all the ingredients in a blender or kitchen robot (like Thermomix). Blend until the mixture is smooth and contains no lumps. Move the mixture to a pan and start to heat on a low setting. Let the mixture simmer until it has thickened and starts to develop a “stretchy” consistency (comparable to melted cheese). Pour the mixture to a mould, let cool slightly before storing it in a fridge overnight.

Vegan butter:

Recipe: coconut oil, aquafaba (chickpea food-can), sunflower oil, apple cider vinegar, salt

Melt the coconut oil on a low heat, and let cool slightly. Add the sunflower oil to the coconut oil. Place the aquafaba into a bowl, preferably narrow and high rising, with the apple cider vinegar and salt. Use an immersion blender to blend the aquafaba and slightly whip it. Slowly add the oil mixture to the aquafaba, while continuously blending. Place the mixture into a bowl and let it set in the fridge overnight.

Results

First step

Chickpeas

The whipped egg white was first compared to the whipped chickpea moisture derived from food cans (available in Colruyt). It is clear that the reference (egg white) foams very well, and has high stability (89,74%), which is of course the reason why it is used in so many recipes. However, due to foam instability over time, a moisture layer (liquid fraction) is generated after a period of 60 min at room temperature.

Table 2.2: Overview of the whipped period, foaming capacity, stability, firmness, and airiness of whipped egg white (as a control) and 3 versions of whipped chickpea moisture fluid, ALL derived from commercially available food-cans.

	Whipped Egg White	Whipped chickpea moisture fluid (food-can)	Whipped reduced chickpea moisture fluid (food-can) (57,6%)	Whipped chickpea moisture fluid (food-can) after freezing/thawing
Whipping period	2 min	2 min	2 min	2 min
Foaming ability (FA%)	550%	500%	400%	500%
Volume Stability (VS%)	89,70%	92%	100%	83,30%
Firmness	very sturdy	very sturdy	sturdy	very sturdy
Firmness after 60 min (at room temp.)	moisture layer on bottom, rest of foam keeps sturdy	moisture layer on bottom, rest of foam keeps sturdy	keeps sturdy	moisture layer on bottom, rest of foam keeps sturdy
Airiness	very airy	very airy	very airy	very airy

The whipped chickpea moisture from food cans shows very similar foaming capacity, stability and firmness, and is comparable with the characteristics of the egg white reference. It also shows a low amount of a liquid fraction on the bottom resulting from foam breaking (foam instability over time) after 60 min on room temperature. For many homemade recipes, this will (similar to egg white) not be a problem. If it is a problem on more industrial production levels, it can be fixed by reducing the chickpea moisture fluid (by 57,6%) before whipping. In this case the foaming capacity is slightly diminished, but the stability is increased to 100%, a sturdy firmness is caused and there is no moisture layer on the bottom at all after 60 minutes. If on an industrial matter, the whipped chickpea moisture would be frozen and thawed, the product is still whippable and most characteristics are unchanged. Pictures of the experiments are shown in Figure 1.

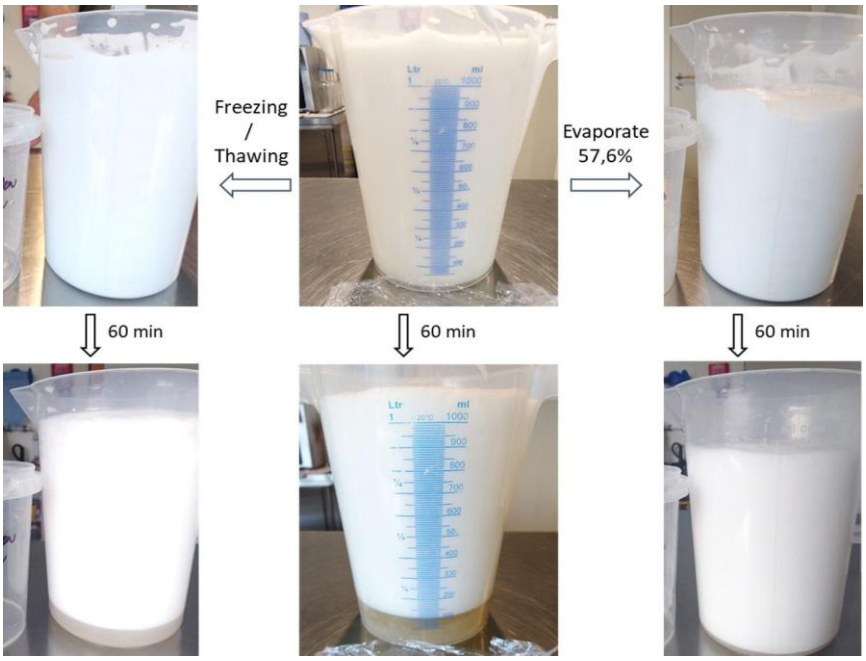

Fig. 2.5: Pictures of the whipped chickpea moisture fluid derived from food-cans. In the middle is the original version. Left the same version, but after freezing/thawing, and right the 57,6% evaporated version.

There was an attempt to produce cooking fluid at ILVO by cooking chickpeas that are commercially available in the supermarket. The chickpeas were soaked overnight, and afterwards cooked 1,2 kg of soaked chickpeas in 3L of water for 1,5 hours, and during cooking, 500 ml of water was additionally added.

Table 2.3: Overview of the whipped period, foaming capacity, stability and firmness of whipped egg white (as a control) and 3 versions of whipped chickpea cooking fluid, All produced in the kitchen.

	Whipped Egg White	Whipped Cooking fluid (dry chickpeas) (after 90 min. cooking)	Whipped reduced cooking fluid (dry chickpeas) (36%)
Whipping period	2 min	No whipping (3 min) => foam, but fluent	Whipping = ok (5 min + adding lemon juice)
Foaming ability (FA%)	550%	-	51,50%
Volume Stability (VS%)	89,74%	-	87,90%
Firmness	very sturdy	-	not sturdy
Firmness after 60 min (at room temp.)	moisture layer on bottom, rest of foam keeps sturdy	-	moisture layer on bottom, not sturdy

In the previous saponins-related research, we noticed that the saponins-content of the cooked chickpeas was approximately 1/3 of the saponins-content available in that of the moisture fluid from a food can. We now notice that the whipping capacities are as well a bit weaker in the kitchen-made cooking fluid when compared to the food can liquid. After whipping it for 3 min, there was some foam, but it was not fluent or sturdy at all. The cooking fluid was reduced (for 36%) and some lemon was added to increase the stability. This improved the whipping effects. However, when we compared the foaming capacity (51,50%) with that of egg white (550%) and food-can excess liquid (500%), it was still quite weak. The stability itself was quite good (87,90%), but the firmness was not. And even in the reduced cooking fluid, there was a liquid fraction layer on the bottom.

It thus seems like only the moisture fluid derived from food-cans shows similar foaming capacity, stability and firmness, as the whipped egg white. Therefore, we also conducted an additional test measuring the hardness using the Texture Analyzer (Lloyd instruments) on the food-can moisture (Figure 2.6).

Fig. 2.6: Hardness measurement using Texture Analyzer (Lloyd instruments). It is clear the hardness of Egg white is significantly higher than those of chickpeas (food-can) and chickpeas (kitchen).

Although foaming capacity and stability of the whipped food-can liquid is very comparable with those of egg white, the measure hardness of all tested samples is however significantly lower.

Soy beans

There were no food-cans of soybeans available. Therefore, we only produced cooking fluid ourselves by cooking soybeans available in the supermarket. We cooked 1,2 kg of soybeans (over nightly soaked) for 120 min in 3 l of water, and additionally added 1 l of water during cooking. We then analysed the original cooking fluid, the reduced version of cooking fluid, and also the original cooking fluid after freezing/thawing.

Table 2.4: Overview of the whipped period, foaming capacity, stability, firmness, of whipped egg white (as a control) and 3 versions of whipped soy beans cooking fluid, ALL produced in the kitchen.

	Whipped Egg White	**Cooking fluid (soy beans) (after 120 min. cooking)**	**Whipped reduced cooking fluid (soy beans) (45,5%)**	**Whipped soy beans cooking fluid after freezing/thawing**
Whipping period	2 min	2 min	2 min	2 min
Foaming ability (FA%)	550%	500%	550%	530%
Volume Stability (VS%)	89,74%	71,70%	84,60%	61,90%
Firmness	very sturdy	sturdy (small bubbles)	sturdy (small bubbles)	sturdy (small bubbles)
Firmness after 60 min (at room temp.)	moisture layer on bottom, rest of foam keeps sturdy	moisture layer on bottom, rest of foam not sturdy anymore. Bigger bubbles	moisture layer on bottom, rest of foam not sturdy anymore. Bigger bubbles	moisture layer on bottom, rest of foam not sturdy anymore. Bigger/small bubbles

The quality of the whipped kitchen-made cooking fluids is surprisingly better than those of the kitchen-made cooking fluids of chickpeas. They all have a strong foaming capacity comparable to those of egg white. Stability is a little bit weaker, although it is quite acceptable, especially for the whipped reduced cooking fluid. The firmness is sturdy at the start, although they do contain small bubbles of air. After 60 min. However, all of the whipped cooking fluid samples have a moisture layer on the bottom, and even worse, the foam itself is not sturdy anymore, and the small bubbles have evolved into bigger bubbles.

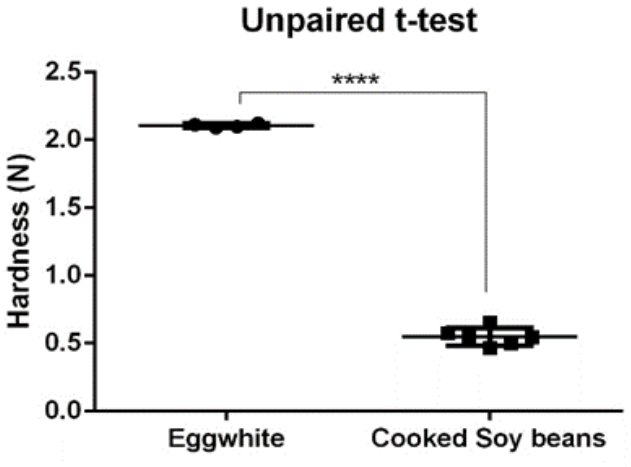

Fig. 2.7: Hardness measurement using Texture Analyzer (Lloyd instruments). It is clear the hardness of Egg white is significantly higher than those of soybeans (kitchen).

The measured hardness of whipped soy beans is significantly lower than those of whipped egg white.

Protein measurement in Aquafaba

These are the results of the Kjeldahl-method, used to detect the amount of organic nitrogen present in the freeze-dried powders of the various 'aquafaba' fluids. We then calculated the amount of proteins present in the powder, and further calculated the amount of proteins present in the 'aquafaba' fluid itself.

Table 2.5: Kjeldahl-results showing the amount of organic nitrogen, and the calculated amounts of protein in the freeze-dried powder and cooking water (aquafaba)

Fluids	Total N (g/100g)	Protein (x6,25) (g/100g)	Moisture content freeze-dried powder (g/100g)	Moisture content cooking water (g/100g)	Protein in cooking water (g/100g)
Chickpeas - kitchen	4,06	25,38	3,58	94,7	1,3
Chickpeas - 'industry'	2,46	15,38	5,28	97,78	0,32
Chickpeas - food can	4,41	27,56	3,32	94,46	1,48
Soybeans - kitchen	3,21	20,06	4,39	92,61	1,42
Soybeans - 'industry'	3,33	20,81	5,05	98,47	0,3

It is clear that the amount of proteins present in the aquafaba-fluids is quite low. In the food can liquid and the kitchen-made cooking fluids, it is between 1,30 and 1,48%. The residual stream (cooking fluid) from the industry was analysed and the protein concentrations there were even lower (0,32 and 0,30%).

Second step: vegan recipes based using whipped cooking fluid

Vegan Chocolate mousse

Three different recipes for vegan chocolate mousse with aquafaba were tested for this research. Due to the results shown above about the foaming capacity, and stability of the several whipped products, we only used the whipped chickpea food-can moisture as aquafaba. This whipped product is most comparable with the commonly used whipped egg white (see Figure 4).

The first recipe included only dark chocolate and aquafaba, and was prepared in two different ways with (recipe 1) and without (recipe 2) apple cider vinegar for stabilization of the foam. In the second recipe, coconut cream was added to mimic the creaminess of a non-vegan chocolate mousse (recipe 3).

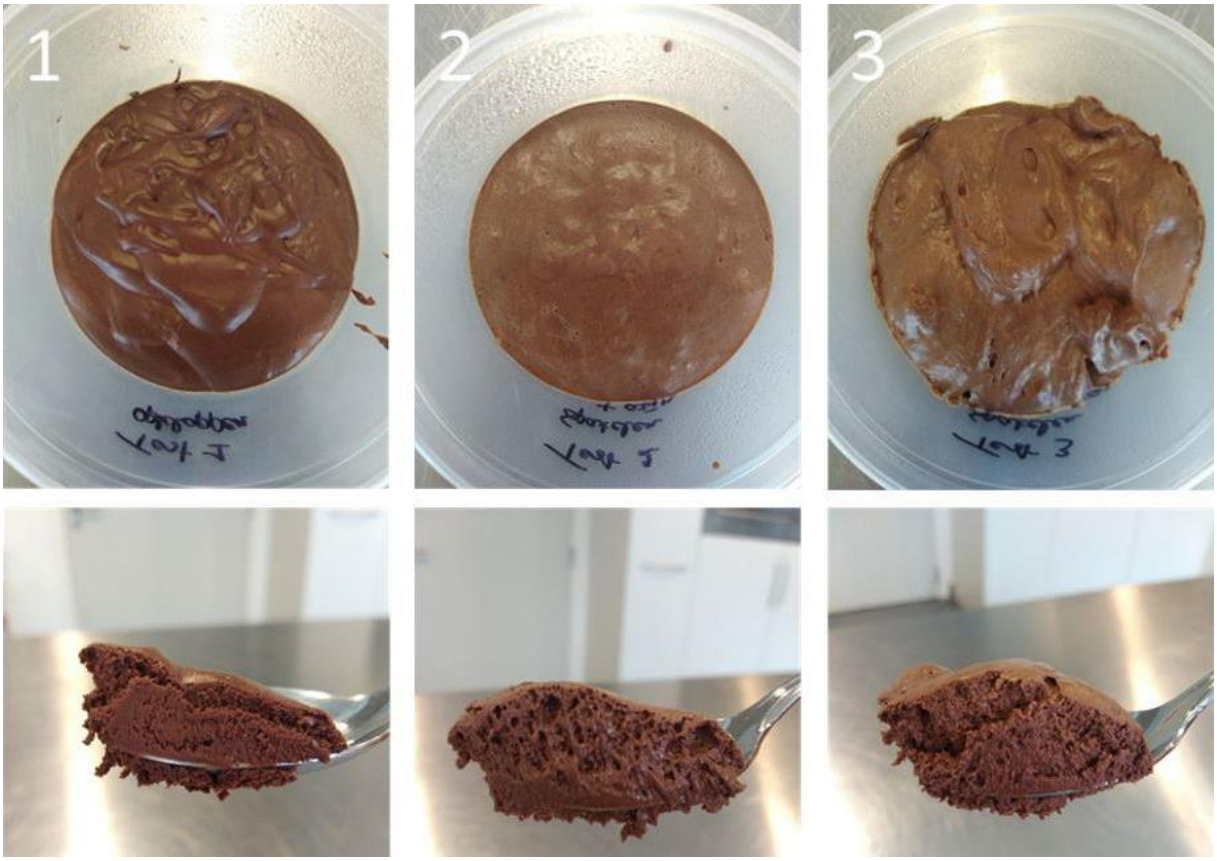

Fig. 2.8: Three recipes tested to produced vegan chocolate mousse. Each time the Egg White is replaced by Whipped Chickpea moisture (aquafaba) derived from canned foods.

The results of the 1st chocolate mousse recipe give a dense chocolate mixture. All the air was knocked out of the aquafaba foam by the extra mixing step in a kitchen robot. The taste is acceptable, very heavy chocolate taste but there is absolutely no taste of chickpeas present.

The chocolate mousse of the 2nd recipe is an airy chocolate mousse with agreeable taste and texture. It is airier than recipe 1 as the aquafaba foam is added to the melted chocolate more

carefully, not using the kitchen robot. The taste of chickpeas is, comparable to result 1, not present either.

The result of the 3rd recipe, using the coconut milk (butter), is also an airy chocolate mousse, slightly denser compared to recipe 2. The coconut cream seemed to add an off-taste, but is not recognizable as coconut. Nevertheless, the taste of recipe 3 is overall acceptable.

Vegan Cheese preparation

As a savoury alternative to the commonly used sweet recipes including aquafaba, we also tried to create a vegan cheese preparation, to be used like a cream cheese or mozzarella. The first recipe has a base of cashew nuts, which are a popular ingredient in vegan cuisine to accomplish a creamy texture (recipe 1). Consequently, the same recipe was used, replacing cashew nuts with one of the legumes used in the previous tests (recipe 2, recipe 3). For the aquafaba used in the cheese preparation, we always used the kitchen-made cooking fluid of commercially available soybeans (Greeny, Bio-Planet).

Fig. 2.9: Three recipes tested to produce vegan cheese preparations. These recipes are vegan alternatives to the more 'classically known' cheeses like cream cheese or mozzarella, which are all based on animal produced milk.

When we look at the first recipe, the mixture from the mould keeps its shape nicely. It is easy to cut and has an agreeable texture (slightly springy, but not rubbery). The taste is nice, it has a cheesy connotation to it, but does not taste like mozzarella. The texture while chewing his smooth, not grainy or rubbery.

This 2nd recipe was not a success in terms of sensory aspects (texture, flavour). The starchiness of the chickpeas leaded to a texture that was less firm and stretchier compared to recipe 1, although it has to be noted that more tapioca flour was used to obtain the cheese-like stretch during the heating process. Also, the flavour turned out to be earthy, directly relatable to the used legumes.

The 3rd recipe was also not a success in terms of sensory aspects (texture, flavour). The starchiness of the white beans leaded to a texture that was less firm and stretchier compared to recipe 1, although it has to be noted that more tapioca flour was used to obtain the cheese-

like stretch during the heating process. Also, the flavour turned out to be earthy, directly relatable to the used legumes.

Vegan butter

We created the vegan butter and used (amongst other ingredients) coconut oil. We used chickpea food-can fluid as aquafaba.

Fig. 2.10: Vegan butter, based on coconut oil and canned chickpea moisture aquafaba

As actually intended, the vegan butter holds a strong coconut flavour, and it is also (perhaps a bit too) salty. After an overnight resting period in the refrigerator, the butter was still not fully solidified, and a soft texture remained. It could be interesting to use other liquid oils to add a nuttier flavour, yellow colour…

Discussion

In this project, it was intended to create new products by re-using by-products derived from the food industry. In this case the cooking fluid was used from chickpeas and soybeans, from several Flemish Industrial food companies producing vegan food products.

We used this residual waste product, as well as kitchen-made cooking fluid of these beans, and moisture from food cans. The latter is widely mentioned in several vegan groups and fora on the internet, and generally called ‘aquafaba’. Previously, it was shown that the saponins-concentration of all of these products is lower than those of other edible products containing beans, and that the product can thus be considered as very safe.

When comparing the whipping effect, stability and firmness of these products with those of egg white, it is clear that chickpea moisture fluid from food cans has most in common with the egg white. The original version, as well as the reduced, and frozen/thawed versions, have high foaming abilities (400-500%), high stability (83,30-100%) and a sturdy firmness. The whipped structure even remains intact after a period of 60 min. And this could possibly be one of the reasons why the product is so popular on the internet.

On a side note, a specific recipe is used to make the liquid fraction from food cans since it also plays a key role in the conservation of the product (= cooked legumes) and may have a different composition compared to the unprocessed cooking fluids that are collected during the industrial cooking process itself. Recipes are also highly variable amongst canning companies and subjected to strict confidentiality.

Reducing the amount of moisture water, helped to obtain foam with the manual method. Presumably, reducing the water even more would be necessary. If we looked at the cooking fluid of soybeans, the results were remarkably better. Foaming abilities were much higher and also more comparable with egg white. However, if we look at the whipped mixture after 60 min, the foam was not that sturdy anymore. If we look at the saponins and proteins concentrations measured above, it seems as the kitchen-made cooking fluid is very comparable with the cooking water by-product from the industry.

Another remark, if we look at hardness comparisons of whipped cooking moisture and whipped egg white, there is a very strong difference. Structures derived from whipped cooking moisture are less strong than structures derived from whipped egg white. Even if the amount of water is relatively low (e.g.: chickpea food can be moist), the hardness remains low. Although it might be possible to create many vegan recipes in the kitchen (as can be viewed on the internet), it will be different to create food products with a long-lasting firm structure in industrial companies. Further research is definitely necessary.

Several recipes were created in-house. Chickpea fluid from food cans and kitchen-made soy bean cooking water was used, and were able to produce good tasting vegan chocolate mousse, vegan cheese preparation and even vegan butter.

The next step is to further investigate the processing of cooking water from legumes into a stable ingredient with better whipping properties, a higher foaming ability and stability, and a much-longer sturdy firmness. Probably, part of the solution is to further reduce moisture content. As for the hardness of the whipped material, several recipes must be tested in practice on an industrial level.

Finally, considering all the results from this study, it can be concluded that there is definitely some potential in re-using this by-product in the future.

2.3.3. Value-added products from Belgian endive by-products

Developed at ILVO

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ILVO Flanders research institute for agriculture, fisheries and food

Introduction

Belgian endives, sometimes also referred to as the Belgian 'white gold' by local farmers, is a typically Belgian agriculture product with a rich history and tradition. The land surface area used for this crop in Flanders is currently estimated to be about 1.200 hectares. Annually, around 40.000 tons of Belgian endive are produced in Belgium which accounted for a turnover of almost 43 million euros in 2017. But the cultivation and processing of Belgian endives also generates a large number of by-products and food-waste fractions that are currently not optimally utilized. These by-products and food-waste fractions can be divided in two separate categories, the roots and the cleaning waste that is mostly composed of leaves. The forced Belgian endive roots account for 36.000 tons yearly and the cleaning and harvesting waste for about 10.200 tons. A third by-product consists of discards from the sorting and grading processes prior to sale on the fresh market. These discards are typically complete Belgian endives that were harvested and cleaned, yet do not meet the quality standards to be sold or traded because of aberrations in shape, size or colour.

The Belgian endive roots are not traded as such but are mainly used as an ingredient for the production of animal feed, for which the farmer/producer receives a maximum compensation of €10 to €15 per ton.

Belgian endive cultivation starts with sowing in mid-May for growing up the roots. In the autumn period, typically between September and November, the roots are harvested by machines, while the green leaves that are emerging above ground level are automatically cut off simultaneously. This by-product of green leaves is normally left on the field and ploughed in, and therefore not optimally utilised or valorised. Additionally, the waste that is left in the field and ends up in the soil it was cultivated in can also be a cause of excess nutrient enrichment and the release of foul odours resulting from decaying organic material.

After harvesting the roots, they are automatically sorted according to their size. The roots with an ideal width between 3 and 5 cm are selected; the others cannot be used for the next step in the cultivation process and are discarded. The deviating roots form a very different kind of by-product compared to the green leaves that were discarded in the field earlier. Various applications can therefore be sought for this. The roots that pass the quality control are used to force the growth of the endive, or are stored to be forced at a later time.

Traditionally root forcing was obtained by planting the roots in soil and covering them with a thick layer of earth so that the shoots don't develop chlorophyll and remain whitish until

harvest. In more recent times farmers have been switching to large-scale hydroponics to increase yields and efficiency.

After 3 weeks in the dark, the Belgian endives are ready for harvesting. The largest number of by-products are released during this harvest step. On the one hand, the roots are detached from the heads; on the other hand, the outer leaves and low-quality Belgian endives that are not suitable for sale are removed. The outer leaves and low-quality Belgian endive provide an annual by-product and food-waste fraction of 10,000 tonnes. This waste is often brought back to the land, where it is ploughed into the field, but other valorisation opportunities could also be investigated. For example, they can serve as an ingredient in feed for insect breeding. Within this Interreg 2 Seas BioBoost project, project partner INAGRO reported on their extensive assessment of using insects as a means to process by-products and waste fractions from agriculture products, including Belgian endive roots and leaves, that could not be otherwise valorised for human food applications. For more details we are referring to the INAGRO Entomospeed report.[1]

However, the most important and most constant by-products are the forced roots which represent 36.000 tonnes annually. They are now mainly used as animal feed, but they also still contain many bioactive substances such as sesquiterpene lactones that could be extracted and used for value-added derivative products. Just like the Belgian endive heads, these bitter substances (sesquiterpene lactones) have various biological and pharmacological properties. Gaining more insights into the presence of these bioactive components is a first step in evaluating alternative valorisations to increase the added value of this by-product. In addition to the various bio-active components, the Belgian endive root is also very rich in fibres which can be used in many applications. To use these fibres, however, they must be first purged of bitter substances and other components to obtain a taste and colourless fibre.

The cultivation of Belgian endives is indispensable in Belgium, both in terms of economic importance and cultural and culinary heritage. One of the advantages of Belgian endive is their year-round availability in large amounts, thus enabling the development of mass processing applications and processes for the use for the otherwise discarded by-products that still hold great valorisation potential.

Aims

The aim of this chapter is the valorisation and development of value-added products based on Belgian endives by-products. We explored two cases, each with a unique application that was developed specifically to create new products, tackling the use of unused by-products of Belgian endives and creating a healthy value-added product that is rich in natural fibres.

[1] https://www.bioboosteurope.com/assets/files/Report-Insect-Breeding-.pdf

Valorisation initiatives for Belgian endive by-products

Within BioBoost we explored several valorisation strategies for either class II whole Belgian endives waste streams or by-products from the production process such as the removed outer leaves of the endive heads or the forced roots that are discarded after the harvest. The goal was to create a finished value-added product with a high market value or high appeal to a general public. These initiatives were worked out into three cases:

Case 1:

In the first case we made fibre-rich cookies using a classic recipe, but added different amounts of purified Belgian endive fibres as a replacement of the flour to assess the effects of adding endive fibres on a set of parameters such as colour, hardness, appearance and taste. The development of new products that are rich in natural fibres could open up new marketing opportunities for these unused agricultural by-products.

Case 2:

In the second case we explored the use of Belgian endives heads that were discarded from market sales for aesthetic reasons (selection discards) as a source ingredient to make endive croquettes for the Belgian consumer market. This novel croquette could be a vegetarian alternative for a fried snack that is very popular in Belgium and the Netherlands and known locally as “Bitterballen”. This snack is typically a small ball-shaped croquette filled with a slightly bitter tasting purée containing meat. The natural bitterness of the Belgian endive extract and fibres mimics the taste of the original snack.

Case 3:

In a third case class II Belgian endives heads that were not sold on the fresh market were used to create a pasta filling for fresh raviolis. The Belgian endives were supplied as whole endive heads, and cut in small pieces to be processed. Several trials have led to a recipe for an innovative Belgian endive filling that could be used in fresh uncooked pasta. The microbiological load and shelf-life of the fresh ravioli were tested at ILVO.

Apart from these cases, ILVO also participated in other collaborations where Belgian endive by-products were processed to obtain a base ingredient for high quality value-added products. One example is the creation of a Belgian white boudin sausage with dried chicory and tarragon by the Belgian enterprise ‘Monsieur Boudin’ (https://monsieurboudin.be/) situated in Ghent (Flanders). Monsieur Boudin specialises in the creation of boudin sausages and seeks out innovation by creating interesting mixes of tastes and ingredients. For one of the creations, the ‘Bertrand’ sausage, they wanted to include Belgian endive powder to the sausage to incorporate the unique and slightly bitter taste of the Belgian endives into their product. For this they used the discarded outer leaves of Belgian endives that were supplied by Versalof

(http://www.versalof.be/en/) and attempted to apply a suited drying technique to obtain the powder that could serve as an ingredient.

Fig. 2.11: A Belgian endive boudin sausage, created by Monsieur Boudin. ©Monsieur Boudin 2020.

ILVO tested several drying techniques after which the company Eco-treasures (https://www.ecotreasures.be/en/ecotreasures-home-eng/) took over to dry Belgian endives for the commercial production of Monsieur Boudin. Their Bertrand sausage with Belgian endives is now commercially available.

Case 1: Fibre-rich cookie with Belgian endive fibres

Preparation of the Belgian endive forced root powder

A total of 20 kg of forced Belgian endive roots were washed to remove dirt and debris, after which the head (the part containing the leaves) was removed by cutting it away manually. The root part was then cut into small fragments with an industrial mixer (robot coupe CL50 Ultra) to obtain small pieces of about 2.5 x 2.5 mm. Before the Belgian endive roots were processed into a powder, they were soaked in a boiling kettle (FIREX) with warm water at a temperature of 60 °C for 3 hours. The water was refreshed every hour. This was done to wash out the sugars and bitter substances out of the product. After this step, the remaining product was dried in a multifunctional cabinet (JUMO Imago F3000) at a temperature of 60 ˚C for four hours, until it contained less than 10% moisture. Once dried, the roots were processed into a powder using a Retsch ZM200 grinding mill equipped with a 0.5 mm sieve mesh.

Making cookies with addition of Belgian endive powder

Vegetable cookies with Belgian endive powder were baked using the following recipe:

Ingredient Quantity
Margarine: 101.25 g
Sucrose: 162.0 g
Sodium bicarbonate: 2.25 g
Flour: 193.5 g - dry matter x 100 /% dry matter (converted to 14% moisture)
Distilled water: 27 ml

Recipe (step by step instructions)

1. The flour was sieved when weighing.
2. The timer started at 7 minutes. Margarine, sugar and sodium bicarbonate were mixed at low speed (position 2) in the electric Kenwood kneader with a K kneading hook for 3 minutes. After every minute, the mixture was scraped down and the dough hook was scraped (to minute 4).
3. The distilled water was added.
4. Mixed speed (mode 3) was mixed for 1 minute (up to minute 3).
5. The mixture was then scraped down and the dough hook was scraped again.
6. The medium speed (position 3) was then mixed for 1 minute (up to minute 2).
7. The flour was then added.
8. Mixed at medium speed for 2 minutes (position 3). Every half minute the mixture was scraped down and the dough hook was scraped (to minute 0).
9. The dough was removed from the kneading bowl and divided into portions.
10. The portions were rolled out with a rolling pin at a thickness of 6.2 mm.
11. The cookies were cut to size with a round cookie mold on a baking paper.
12. The pieces of dough were placed on a baking sheet on the baking paper.
13. The cookies were baked for 14 minutes at 185 ° C in a preheated MIWE convection oven, on ventilation 1.
14. The cookies were then placed on a baking sheet on a rack for 30 minutes before cooling at room temperature.

(Barbara Duquenne, March 21, 2019 - oral information)

Part of the flour has been replaced by this powder (0%, 10%, 25% and 50%). The cookie with 0% Belgian endive powder served as the reference. The other cookies containing different percentages of Belgian endive powder were directly compared to this reference cookie. The cookie that most closely matched the reference was compared visually and by taste. To assess whether untrained consumers could notice a difference between the reference cookie (0%) and the cookie with Belgian endive powder (10%, 25% or 50%), an analytical sensory study was conducted. A triangle test was applied for this purpose (Brinkman, 2016).

Sensory examination

The sensory examination was conducted with a minimum of 30 people (N = 30). In order to collect data as correct as possible, tasting took place between 10 a.m. and 12 a.m. The product was tested at room temperature, so that the flavours of the product were highlighted (Brinkman, 2016). The cookies are offered in random order. In addition to the standard triangle test question, which cookie differs from the others other questions have been asked:
- Which cookie is the tastiest?
- Why don't you like the other cookie?
- Are the cookies acceptable in terms of taste?
- Which cookie has the best structure?
- Why does the other cookie have a less good structure?
- Are the cookies acceptable in terms of structure?

Dietary fibres in the Belgian endive powder

The dietary fibre content in the Belgian endive powder was calculated using the Dietary fibre kit. One measurement was performed per sample (N = 1). The procedure that was used was "Megazyme: Rapid Integrated Dietary Fibre Analysis" (Megazyme, 2018). Furthermore, the number of dietary fibres was determined per 100 grams of the product.

Fig. 2.12: shows the images of the baked cookies. There is a noticeable visual difference between the different recipes, with the most striking characters that differ being colour, shape and coherence.

The cookie that contained 25% endive fibres was very crumbly and easily fell apart after baking. It also showed many fissures and cracks. The unbaked dough itself was very crumbly resulting in great difficulties to roll out the dough and form the cookies. Because of the high fibre content, the cookies kept better the initial form that was given to them and therefore resulted in a less broad and higher cookie after baking. Compared to the reference cookie there was also a difference in colouration, with fibre-rich cookies appearing more faded compared to the reference cookies. The cookies with addition of 10% fibres had an appearance in between the reference and the ones with 25%. Although the cookies didn't fall apart due to too much crumbliness, some cracks and fissures could be detected by the naked eye after baking. The cookies retained better their initial shape compared to the reference, but still less than the cookies with 25% fibres.

Additionally, because the cookies with more fibres kept their shape and didn't flatten as much as the reference cookie, the underside also became much darker during the baking, as the heat of the oven plate was distributed over a smaller surface area.

Based on these results, it was decided to test the cookie with 10% Belgian endive powder in the sensory study.

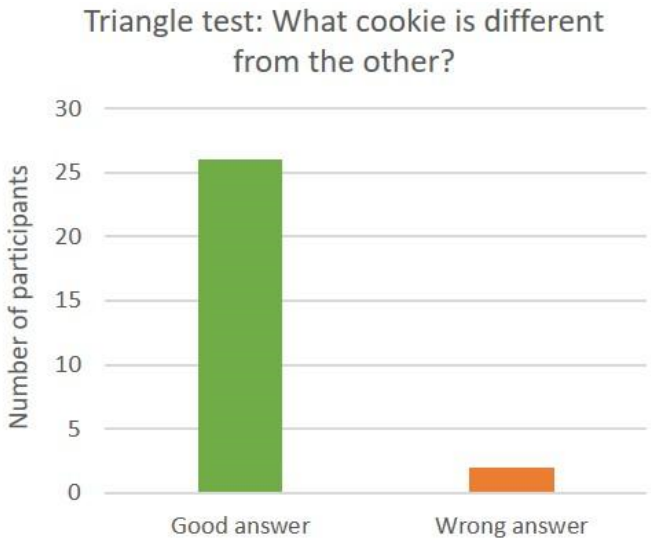

Fig. 2.13: shows the results of the triangular test. Here it can be seen that 26 of the 28 participants answered correctly to the question which cookie differs from the other cookies. Appendix 6 shows the answers to the questions of the triangular test, as they were answered by the participants.

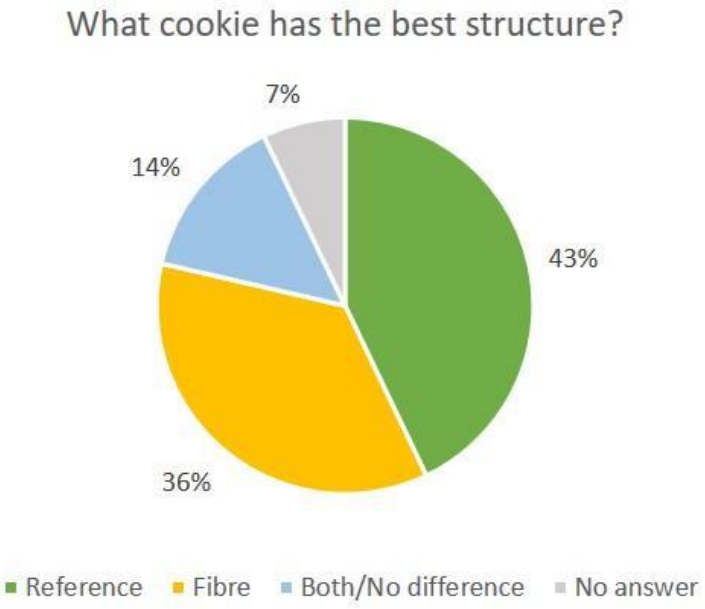

Fig. 2.14: shows the answers for the question "What cookie has the best structure?". It was found that 43% of the participants think the reference cookie has the best structure. 36% of the participants think that the cookie with fibre has the best structure. In addition, 18 out of 28 participants indicated that both cookies were equally acceptable in terms of taste.

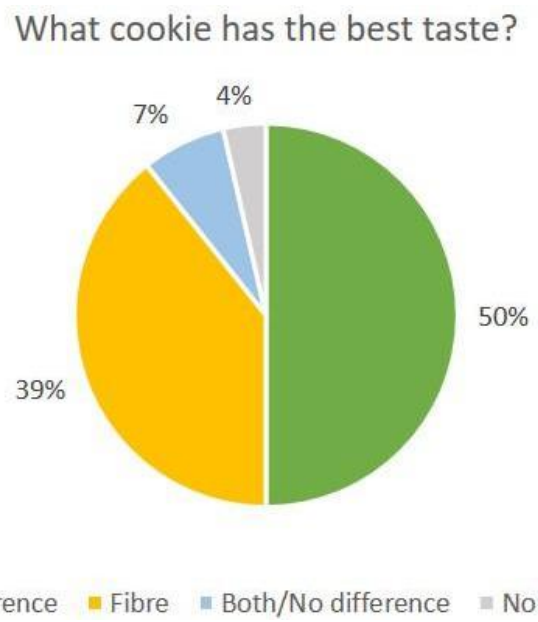

Fig. 2.15: shows the answers to the question which cookie is the tastiest. It turned out that 50% of the participants liked the reference cookie the most. 39% of the respondents said that they found the cookie with 10% fibre the tastiest. In addition, 18 out of 28 participants indicated that both cookies were equally acceptable in terms of taste.

The average weight of a cookie was 8.8 grams. This means that the cookie with 10% Belgian endive powder contained 0.88 grams of Belgian endive powder. The cookie contained 0.76 grams of dietary fibre derived from Belgian endive powder. From the other ingredients, only flour still contains dietary fibre, namely 4 grams per 100 grams (Nevo-online, 2016). Together with the other ingredients, one cookie contains 1.11 grams of dietary fibre. This means that per 100 grams 12.61 grams or dietary fibre is present in the product.

Discussion

The results of baking cookies with Belgian endive fibre have shown that, with the recipe followed, the cookies where 25% flour has been replaced by Belgian endive powder became very crumbly. The dough fell apart as it rolled out and was difficult to shape into cookies. It was therefore decided not to bake cookies with 50% Belgian endive powder. The crumbliness can be because there is not enough binding in the dough, making it very crumbly. Trying a different recipe or adding more moisture could reduce crumbles. The dough and visual aspect of the cookie with 10% Belgian endive powder best matched the reference cookie. This is therefore compared with the reference in the triangles test. In the triangle test, in addition to the normal triangle test question, a number of other questions were asked. At the answer screen, the answer box jumped, so that some questions could not be read or the answer was entered in the wrong box. As a result, results may differ.

The recipe that was used to make the cookies resulted in typical sand-cookies that were quite hard. Adapted recipes to obtain softer cookies can off-course be applied to obtain a variety of cookie-types in which the Belgian endive fibres can be processed.

Conclusions

It can be concluded that the difference between the reference cookie and the cookie with 10% Belgian endive powder is noticeably present. Furthermore, it can be said that both cookies are acceptable in terms of taste and texture. On the cookie with 10% Belgian endive powder, the nutrition claim should be stated as "high in fibre" since 100 grams of the product contains 12.61 grams of dietary fibre (European Commission, 2012). This conclusion is based on the previously found results on the dietary fibre content in Belgian endive powder (Twarogowska, 2019, unpublished results).

Due to a lack of time it was not possible to determine the percentage of dietary fibre in the Belgian endive powder. It is advisable to carry out this analysis for the Belgian endive powder used in order to be able to draw a good conclusion about the number of grams of dietary fibre in a cookie. It is also recommended to carry out further research into the percentage of Belgian endive powder in the cookies, by using other recipes and proportions. The crumbliness of the dough could be reduced by adding more moisture or butter to the dough. Hand-kneading could also help to reduce crumbliness.

Case 2: Using discarded endive heads as a high value ingredient for endive croquettes

In contrast to the previous case, which is putting the focus on the use of discarded forced endive roots, this case focuses on the valorisation of Belgian endive heads and leaves that were discarded for aesthetic reasons during the final post-harvest quality control and selection. These endives deemed unfit for direct sale because they are either too small or slightly deformed. In terms of product quality, they are however equivalent to the endives that are sold as A class endives to the general public.

The pre-research and feasibility assessment for this case was performed during another Interreg project (= Interreg: Food from Food)[1] and was developed in direct collaboration with a farmer[2] of traditional Belgian endives that were cultured in soil instead of aquaponics and a company that produces the croquettes[3].

[1] https://www.foodfromfood.eu/

[2] https://www.rechtvanbijdeboer.be/grondwitloof-van-bij-de-boer-ook-echt-beter

[3] https://gastronello.wixsite.com/gastronello/over-ons

Developing a continuous industrial production process

The aim of this case is to develop for a continuous production process (simulating a large-scale industrial production process) rather than a batch by batch procedure. At the time that this product was developed, no commercial presser was available or had the proper equipment to perform the pressing of the endives to obtain a fraction of juice and a fibre rich press cake. Although many industrial pressing companies are available for pressing, they either don't have the right equipment to press vegetables or the combination that they offer does not include the possibility to sterilise the pressed juice. In most cases a heating unit is coupled to the presses that allows for pasteurisation of the obtained juice, however pasteurisation is insufficient in the case of Belgian endive juice that is characterised by a relatively high pH. Sterilisation is a necessity, yet this combination of pressing and sterilising is not commercially available. To bypass these limitations of the industry, ILVO provided support to the grower and producer by making its infrastructure available, with permission from the Belgian Food safety agency (FAVV) to produce food-safe batches for use to make the croquettes.

Initially the raw product needs to be cleaned to avoid debris and remains of earth to end up in the press and contaminate the end-product. After cleaning, the endive heads and leaves are cut and are transported to a multicut which cut the endive to a purée. Next, the puree is transported to a vacuum press (VaculIQ®) to obtain two fractions, a liquid juice fraction and a fibre-rich press cake.

Step 1: Washing the endive heads and leaves

Since the endives were produced using the traditional underground method, they often still contain some remains of dirt and sand, and must be washed/cleaned to remove all unwanted debris and remains of earth sticking to the leaves. To achieve this the endives are briefly sprayed with water in a tub, the excess water is then drained and removed, after which the endives are cut in smaller pieces.

Washing the whole endive head has proven problematic, with large amounts of water remaining inside the endives. This washing step was significantly improved by cutting in half (longitudinally) the endive heads prior to washing. This manual step is however more time consuming and labour intensive, particularly for larger batches.

Step 2: Cutting, washing and transport

The Belgian endives leaves must first be cut in small pieces before entering the press. They are first collected in a container and transported via a conveyor belt that is equipped with a water spraying installation to wash the leaves as they are being transported to the Dicer. The Dicer is equipped with rotating blades and a transport shrew, both cutting the endives in small pieces and pushing the mix towards the entry of the VaqulIQ® press (Fig. 2.16).

Fig. 2.16: Washed endive leaves are transported to the mixer (left), the mixer cuts the leaves to obtain a pulp (middle), the mixed leaves are pumped to the VaquIIQ® directly after mixing (right).

Step 3: Pressing under vacuum

The VaquIIQ® press separates the mixed endive leaves to produce two distinct fractions. On one side the juice that is pressed is collected in a vacuum vessel and can be transported to the UHT line for the heat-treatment and filling in bag-in-boxes. On the other side a press-cake that contains all the fibres and hard material is produced and can be collected.

Fig. 2.17: Whole Belgian endive discards (with visual defects) that serve as start material (top left), the liquid fraction (endive juice) with typical reddish colouration due to oxidation (lower left), and the solid fraction (= press cake) with all the solids (including fibres) that remain after pressing (right).

Step 4: Treatment of the press products

The juice can be pasteurised or sterilised and the press cake was stabilised by heat treatment (Fig. 2.18). Pasteurisation of the endive juice proved to be insufficient to guarantee conservation, but sterilisation worked well.

Croquettes from discarded Belgian endive leaves

The creation of an endive croquette was done in close collaboration with the family company Gastronello, who specialises in the creation of all sorts of croquettes. The cutting-edge innovative techniques used to obtain the press cake fractions and juice under vacuum pressing at ILVO are not yet available for commercial large-scale production. Therefore, ILVO and Cools, with official licensing and under supervision of the Belgian Food Safety authority FAVV, collaborated to develop a small-scale commercial production for the creation of the basic ingredients made from Belgian endive leaves for the croquettes.

Fig. 2.18: Press cake coming out of the VaquIIQ® (left), dried press cake material (middle), and dried press-cake being cut to obtain fine material using a Seydelmann vacuum-cook-cutter.

Fig. 2.19: The final realisation, an innovative Belgian endive croquette, made from by-products (discarded outer leaves) of the Belgian endive production.

Case 3: Using discarded endive heads as a base ingredient for the creation of fresh Raviolis with a Belgian endive filling.

Introduction

Belgian endives that were rejected for fresh market sales because of visual defects (open crop, spots or damaged), but were otherwise indistinguishable from the fresh market class I selected endives, can still be used as ingredients to prepare a variety of convenience foods or semi-processed products that can be further processed to obtain ready-to-eat products.

The Belgian company Pastati tested the use of class II Belgian endive heads as a base ingredient to prepare the filling for freshly prepared raviolis. Pastati specialises in the creation of sustainable and eco-friendly pasta meals that are prepared in an artisanal fashion with fresh products, but also as much as possible with high quality by-products from agriculture and horticulture. The small-scale production is supported by a close collaboration with a sheltered workshop company (trans. maatwerkbedrijf in dutch). An overview of their product line-up can be consulted on their website: https://www.pastati.be/

The ravioli that Pastati makes are a semi-finished product that is ready for final preparation by the customer, with fresh uncooked pasta and pre-heated but not cooked fillings that are made from selected vegetables. Their recipes are not fixed but can be changed according to the availability of the ingredients, allowing them to quickly adapt to changes in seasonal vegetables and by-products that are not always abundant throughout the year.

Fig. 2.20: The raw ravioli pasta with a filling made with class II Belgian endives made by Pastati

Aims

In this chapter we explore the conservation potential for these fresh semi-finished products and formulate some recommendations based on the microbial analysis performed on them.

Methods

Fresh class II Belgian endive heads were obtained by ILVO directly at a local grower and delivered at Pastati for the creation of the pasta filling. Since the use of Belgian endives was a new ingredient for the fillings, preliminary tests were performed at Pastati to evaluate the

potential of the endives to be used for the filling. Several procedures were tested prior to the development of the final recipe.

The ravioli was then transported immediately after preparation to ILVO to investigate the conservation potential under atmospheric packaging and under MAP sealed packaging. The evolution of spoilage inducing bacteria and fungi was monitored for the duration of 7 days.

Results

Preliminary testing of the Belgian endive mix

The following tests were done by Pastati prior to the final preparation of the filling for the fresh Ravioli's.

- The class II Belgian endive heads were cleaned with water, then cut in large pieces and cooked in the oven at 200°C until ready. The pieces were mixed when still warm in a Thermomix until no pieces remained.

  result: The obtained mix is too soggy, there is an excess of water in the mixture, it can't be used for the pasta.

- The class II Belgian endive heads were cleaned with water, then cooked in a cooking pot covered with baking paper. The pieces were mixed when still warm in the Thermomix.

  result: The obtained mix is too soggy, there is an excess of water in the mixture, it can't be used for the pasta.

- The class II Belgian endive heads were cleaned with water, then mixed raw in the thermomix during 10 mins at 80°C on position 1.5.

  result: The obtained mix is too soggy, there is an unpleasant looking discolouration.

- The class II Belgian endive heads were cleaned with water, then mixed raw in the thermomix for 10 mins, then stoved shortly in a pan.

  result: The obtained mix has an unpleasant looking discolouration and is unpalatable (bad taste).

- The class II Belgian endive heads were cleaned with water, then cut in large pieces and cooked in the oven at 200°C until ready. The pieces were mixed when still warm in a Thermomix, using a mixing function on position 2 for 30 seconds.

  result: The obtained mix is possibly usable, although there are some pieces left, the pieces might be too big for use in the pasta machine. This method was selected for the final preparation of the filling for the fresh ravioli and provided satisfactory results.

Future perspectives, towards a zero-waste valorisation

In an ideal scenario, working with by-products would result in a zero-waste cycle where all of the generated by-products can be used to generate new commercial processed products. In the case of the Belgian endive croquettes, the leaves are pressed to obtain juice and a fibre-rich fraction. However, although both fractions are used in the creation of the final product, there is an excess production of juice that remains unused for this application. This juice, which becomes on its turn a by-product of the pressing process, can further be processed, for example by evaporation or drying to obtain a concentrate of powdered product that still holds all the important components. For BioBoost, ILVO attempted to spray-dry the Belgian endive leave juice obtained after pressing, but lab-scale tests failed to produce the desired results. Alternatively, the excess juice that is produced during the pressing could find other applications that don't involve drying such as an additive to drinks, lemonades or other beverages.

2.3.4. Pointed sweet bell pepper jams, jellies and ketchups

Developed at ILVO in partnership with the food company "Zoete Potjes"[1]

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ILVO
Flanders research institute for agriculture, fisheries and food

Introduction

The pointed sweet bell peppers that were used to create value added preparations were class 2 discards. In terms of general quality and taste, these by-products are virtually identical to the class 1 selected pointed sweet bell peppers for the fresh market. However, the class 2 didn't make it through the final sorting and visual quality checks prior to sale for the fresh market. They were discarded mainly because of superficial damage to the skin (abrasions, dents, spots and bruises), or an atypical shape (folded, wrinkled, etc…) that would make them less attractive to the mainstream customer. These are subjective quality control parameters that don't influence the taste and colour of the final product once the peppers are being processed, and thus several applications for discarded pointed sweet bell peppers were applied to create sweet pepper jams and jellies to valorise the discards for the local market without passing through the large retailers (preferably short chain applications, from producer to customer).

Methods

Several in-house recipes were developed at ILVO to create jams and jelly based on pointed sweet bell peppers, with or without the addition of other by-product ingredients.

Additionally, in collaboration with the Belgian company "Zoete Potjes", a pointed sweet bell pepper ketchup and a chutney with pieces of pointed sweet bell peppers was developed, targeting a possible future commercialisation after rigorously testing the product. "Zoete Potjes" specialises in the use of by-products for the creation of innovative products and original taste combinations that are otherwise not available for the consumer market.

A full analysis of the nutritional value of the used pointed sweet bell pepper fraction (class 2) was performed (table. 2.5).

The viscosity of the tomato ketchups and the sweet bell pepper ketchups was measured and compared to a commercial reference sample using an Anton Paar modular compact rheometer model MCR302 equipped with a B-CC27 measuring cylinder. Each sample was measured three times using a different sub-sample to compare homogeneity and reproducibility within the sample.

[1] https://www.zoetepotjes.be/

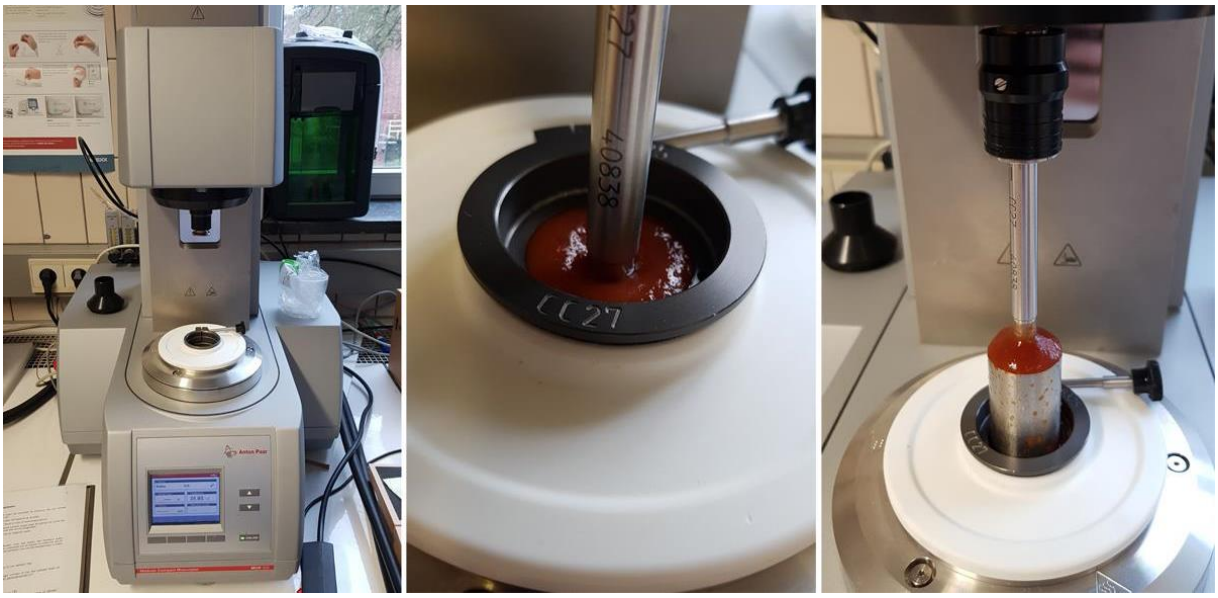

Fig. 2.21: Using the Anton Paar rheometer with a CC27 rotating measuring cylinder, during the measurement (middle) and when retracting with the measuring cylinder head partially visible (right).

Results

Nutritional composition

Table 2.5: Nutritional values for red and yellow pointed sweet bell peppers (Analysis performed by Servaco for ILVO - BioBoost)

Parameter	Pointed bell peppers		Unit
	Red	Yellow	
Ash	0,37	0,39	g/100g
Calcium	< 0,007	< 0,007	g/100g
Caloric value (kcal)	30	27	kcal/100g
Caloric value (kJ)	127	114	kJ/100g
Dry product	9,1	7,6	g/100g
Protein	0,81	0,9	g/100g
Phosphor	0,02	0,01	g/100g
Iron	< 10	< 10	mg/kg
Potassium	0,186	0,194	g/100g
Carbonhydrates	5,5	4,4	g/100g
Magnesium	0,009	0,01	g/100g
Manganese	< 5	< 5	mg/kg
Sodium	< 0,01	< 0,01	g/100g
Soluble sugars (glucose)	6,7	4,8	g/100g
Fat (total)	0,2	0,28	g/100g
Vitamin C (Ascorbin acid)	207	175	mg/100g
Moisture	90,9	92,4	g/100g
Nutritional fibres	2,4	1,6	g/100g
Salt (in NaCl equivalenets)	<0,025	< 0,025	g/100g

Jams and jellies based on pointed sweet bell peppers

- *Red pointed sweet bell pepper jam*

Ingredients: Red Pointed sweet bell pepper, refined sugar, Imperial Pec Plus [1]

Recipe:

- ✓ Mix the cleaned red pointed sweet bell pepper with a mixer (thermomix)
- ✓ Add sugar
- ✓ Gently boil the mixture of peppers and sugar for ± 20 minutes
- ✓ Add Pec Plus and let it boil for about 2 minutes
- ✓ The jam is ready for use after cooling down

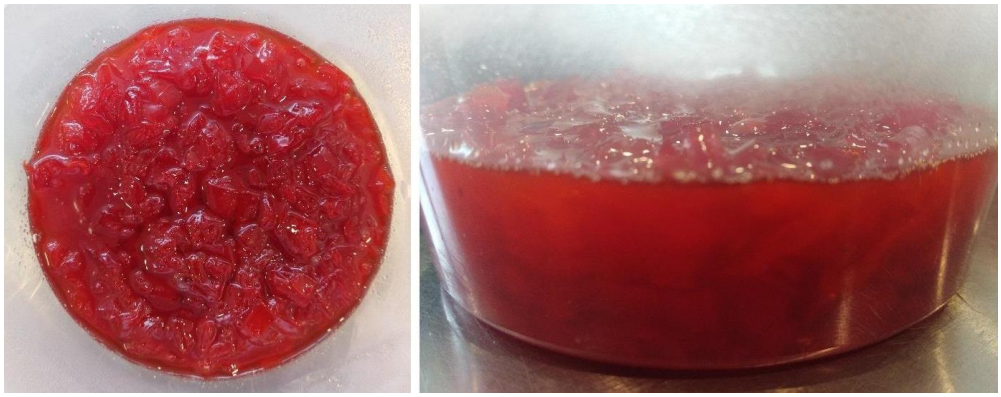

Fig. 2.22: Red pointed sweet bell pepper jam

- *Yellow pointed sweet bell pepper jam*

Ingredients: Yellow Pointed sweet bell pepper, refined sugar, Imperial Pec Plus

Recipe:

- ✓ Mix the cleaned yellow pointed sweet bell pepper with a mixer (thermomix)
- ✓ Add sugar
- ✓ Gently boil the mixture of peppers and sugar for ± 20 minutes
- ✓ Add Pec Plus and let it boil for about 2 minutes
- ✓ The jam is ready for use after cooling down

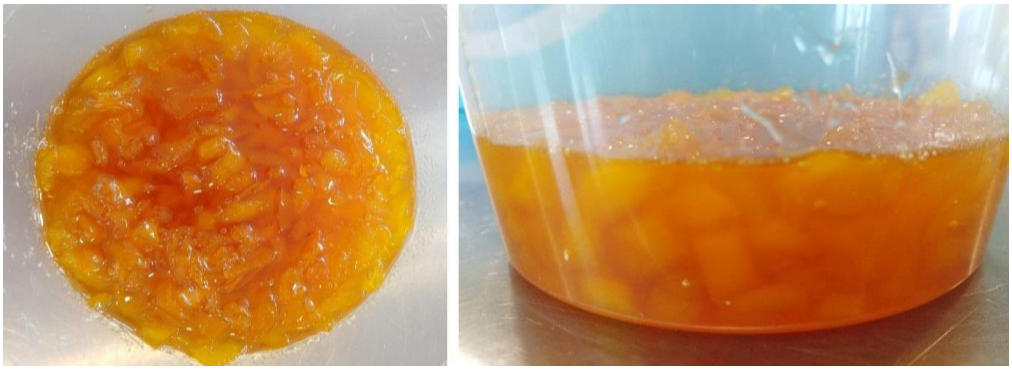

Fig. 2.23: Yellow pointed sweet bell pepper jam

[1] https://imperialbaking.be/nl/producten/pec-plus

- *Yellow pointed sweet bell pepper jam with courgette and Ginger root*

Ingredients: Yellow pointed sweet bell pepper, courgette, refined sugar, fresh ginger root, Imperial Pec Plus

Recipe:

- ✓ Cut the cleaned yellow pointed sweet bell pepper in small blocks/pieces
- ✓ Grate the courgette to obtain small pieces
- ✓ Add sugar
- ✓ Grate the ginger root
- ✓ Mix everything and boil it for about 20 minutes
- ✓ Add Pec Plus and let it boil for 2 additional minutes
- ✓ The jam is ready for use after cooling down

Remarks: Dose the ginger according to taste, be aware that the ginger tends to quickly become the dominant taste, overshadowing the other ingredients. It became apparent during tasting sessions with a panel of untrained testers that the personal preference for ginger can be very person-dependent. Some testers had a clear preference for less ginger, while others thought that it was perfectly balanced when more ginger was added.

Fig. 2.24: Yellow pointed sweet bell pepper jam with courgette and ginger root

- *Red pointed sweet bell pepper jam with courgette*

Ingredients: Red pointed sweet bell pepper, courgette, refined sugar, Imperial Pec Plus

Recipe:

- ✓ Cut the red pointed sweet bell pepper in small blocks/pieces
- ✓ Grate the courgette to obtain small pieces
- ✓ Add sugar
- ✓ Grate the ginger root
- ✓ Mix everything and boil it for about 20 minutes
- ✓ Add Pec Plus and let it boil for 2 additional minutes
- ✓ The jam is ready for use after cooling down

Fig. 2.25: Red pointed sweet bell pepper jam with courgette

- *Yellow pointed sweet bell pepper jelly*

Ingredients: yellow pointed sweet bell pepper, refined sugar, Imperial Pec Plus

Recipe:

- ✓ Cut the yellow pointed sweet bell pepper in small pieces
- ✓ Add refined sugar and warm this mixture for about 20 minutes
- ✓ Add Pec Plus and let it boil for another 2 mins
- ✓ Filter out the pieces of pointed sweet bell pepper and retain the liquid fraction.

- *Red pointed sweet bell pepper jelly*: Identical to the red pointed sweet bell pepper jelly recipe but with red peppers instead.

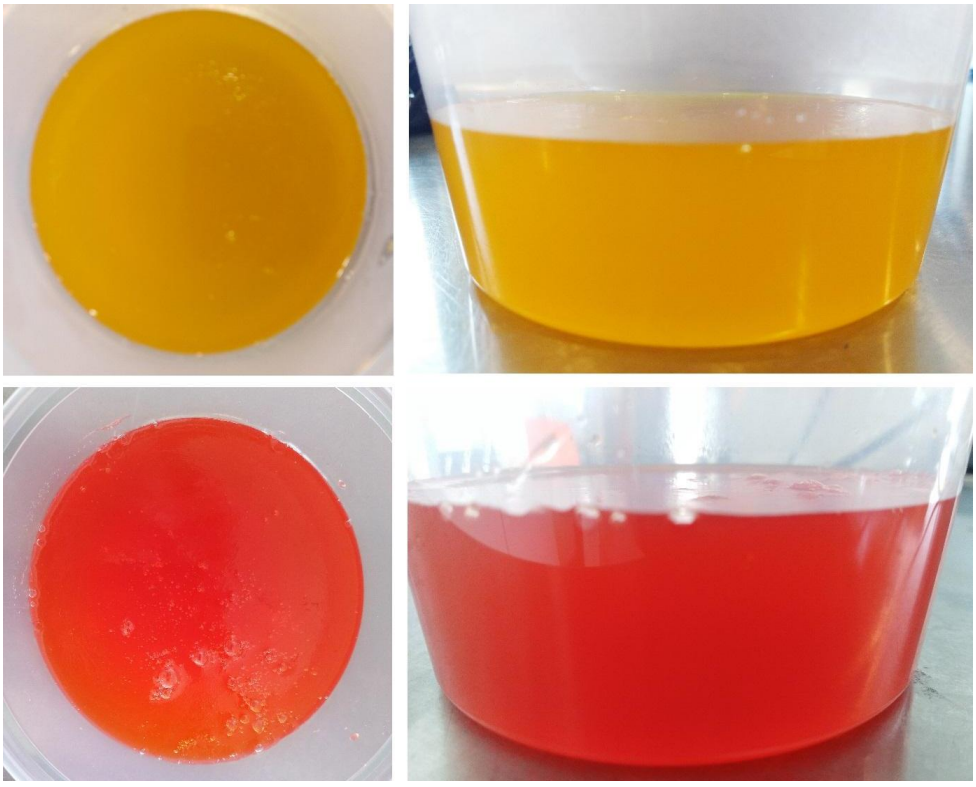

Fig. 2.26: Jelly from red or yellow pointed sweet bell peppers from class 2 pointed sweet bell peppers.

Pointed sweet bell peppers ketchup and chutney

Two recipes were developed by the Belgian company 'Zoete Potjes' and tested for the creation of a ketchup based on tomato and pointed sweet bell pepper, and a chutney with the addition of pointed sweet bell peppers. Two variants were made for the tomato ketchup and sweet bell pepper ketchup, one had only maizena as a binder, the other variant used both maizena and pectin.

Recipe for tomato-ketchup (with class II tomatoes)

For confidentiality reasons, only a crude listing of the used ingredients is provided.

- ✓ Tomato concentrate purée
- ✓ Fresh Tomatoes (class II by-product)
- ✓ Maizena
- ✓ Pectin (Pec Plus) -> only for the variant with maizena + Pectin.
- ✓ Onion
- ✓ Garlic
- ✓ Sugar (refined)
- ✓ Balsamico (Balsamic vinegar)

Recipe for ketchup with pointed sweet bell pepper

For confidentiality reasons, only a crude listing of the used ingredients is provided.

- ✓ Tomato concentrate purée
- ✓ Fresh Tomatoes (class II by-product)
- ✓ Sweet bell peppers (class II by-product)
- ✓ Maizena
- ✓ Pectin (Pec Plus) -> only for the variant with maizena + Pectin.
- ✓ Onion
- ✓ Garlic
- ✓ Sugar
- ✓ (Balsamic vinegar)

Recipe for chutney with pointed sweet bell pepper

For confidentiality reasons, only a crude listing of the used ingredients is provided.

- ✓ Tomato concentrate purée
- ✓ Sweet bell peppers (class II by-product)
- ✓ Pectin (Pec Plus)
- ✓ Sugar
- ✓ Onion
- ✓ Garlic
- ✓ Ginger root

The procedure for making the ketchup and the chutney are very similar and follow the same basic steps except that for the ketchup an additional sieving step is used to remove all chunks

and clumps from the mixture after it has been mixed and heated. The proportions of each ingredient into the preparation are also different.

After cutting the onions and tomatoes (and additionally red sweet bell peppers in case of sweet bell pepper ketchup) all ingredients are heated and regularly stirred in a large pot. When ready the mixture is transferred to a thermomix device where the mixture is thoroughly mixed to eliminate all large chunks and particles. Then the mix is sieved over a fine mesh kitchen sieve and immediately transferred to air sealed glass jars. The ketchups are still very hot at the time of filling the jars, guaranteeing a sterile environment without germs and a long storage time.

These recipes were applied to obtain the following ketchups and chutney:

- Tomato ketchup with balsamic vinegar + maizena
- Tomato ketchup with balsamic vinegar + maizena and pectin
- Tomato ketchup with balsamic vinegar and cherry vinegar + maizena
- Tomato ketchup with balsamic vinegar and cherry vinegar + maizena and pectin
- Sweet bell pepper ketchup + maizena
- Sweet bell pepper ketchup + maizena and pectin

Fig. 2.27: A line-up of different novel realisations developed by the company 'Zoete Potjes' in collaboration with ILVO. A: tomato ketchup with balsamic vinegar, B: sweet bell pepper ketchup (darker colour), and C: chutney with pieces of tomato, onions, ginger and pointed sweet bell peppers.

Nutritional analysis

Tables 2.6 → 2.10 give an overview of the nutritional analyses of the ketchups and the chutney that were done by Servaco for ILVO.

Table 2.6: Tomato ketchup with balsamico: maïzena + pectin

Parameter	Value	Unit
Ash	0.63	g/100g
Energetic value (kcal)	60	kcal/100g
Energetic value (kJ)	255	kJ/100g
Dry product	16.3	g/100g
Proteins	0.92	g/100g
Carbonhydrates	13.5	g/100g
Potassium	0.010	g/100g
Dissolved sugars (glucose)	11	g/100g
Total fat	<0.20	g/100g
Fatty acids (mono unsaturated)	-	g/100g
Fatty acids (poly unsaturated)	-	g/100g
Fatty acids (trans)	-	g/100g
Fatty acids (saturated)	-	g/100g
Moisture content	83.7	g/100g
Dietary fibres	1.3	g/100g
Salt (NaCl equivalents)	0.025	g/100g

Table 2.7: Tomato ketchup with balsamico en cherry vinegar: maizena + pectin

Parameter	Value	Unit
Ash	0.71	g/100g
Energetic value (kcal)	65	kcal/100g
Energetic value (kJ)	274	kJ/100g
Dry product	17.7	g/100g
Proteins	0.98	g/100g
Carbonhydrates	14.4	g/100g
Potassium	0.012	g/100g
Dissolved sugars (glucose)	12.4	g/100g
Total fat	<0.20	g/100g
Fatty acids (mono unsaturated)	-	g/100g
Fatty acids (poly unsaturated)	-	g/100g
Fatty acids (trans)	-	g/100g
Fatty acids (saturated)	-	g/100g
Moisture content	82.3	g/100g
Dietary fibres	1.6	g/100g
Salt (NaCl equivalents)	0.03	g/100g

Table 2.8: Pointed sweet bell pepper ketchup: maïzena + pectin

Parameter	Value	Unit
Ash	0.79	g/100g
Energetic value (kcal)	80	kcal/100g
Energetic value (kJ)	341	kJ/100g
Dry product	20.5	g/100g
Proteins	2.28	g/100g
Carbonhydrates	17.1	g/100g
Potassium	0.024	g/100g
Dissolved sugars (glucose)	15.4	g/100g
Total fat	0.32	g/100g
Fatty acids (mono unsaturated)	-	g/100g
Fatty acids (poly unsaturated)	-	g/100g
Fatty acids (trans)	-	g/100g
Fatty acids (saturated)	-	g/100g
Moisture content	79,5	g/100g
Dietary fibres	<1.0	g/100g
Salt (NaCl equivalents)	0.060	g/100g

Table 2.9: Chutney

Parameter	Value	Unit
Ash	0.49	g/100g
Energetic value (kcal)	130	kcal/100g
Energetic value (kJ)	550	kJ/100g
Dry product	31.4	g/100g
Proteins	0.95	g/100g
Carbonhydrates	26.3	g/100g
Potassium	0.013	g/100g
Dissolved sugars (glucose)	26.0	g/100g
Total fat	2.01	g/100g
Fatty acids (mono unsaturated)	1.4	g/100g
Fatty acids (poly unsaturated)	0.2	g/100g
Fatty acids (trans)	<01	g/100g
Fatty acids (saturated)	0.4	g/100g
Moisture content	68.6	g/100g
Dietary fibres	1.7	g/100g
Salt (NaCl equivalents)	0.033	g/100g

Table 2.10: Reference ketchup: Heinz tomato ketchup

Parameter	Value	Unit
Ash	-	g/100g
Energetic value (kcal)	103	kcal/100g
Energetic value (kJ)	435	kJ/100g
Dry product	-	g/100g
Proteins	1.2	g/100g
Carbonhydrates	23.2	g/100g
Potassium	-	g/100g
Dissolved sugars (glucose)	22.8	g/100g
Total fat	0.1	g/100g
Fatty acids (mono unsaturated)	-	g/100g
Fatty acids (poly unsaturated)	-	g/100g
Fatty acids (trans)	-	g/100g
Fatty acids (saturated)	-	g/100g
Moisture content	-	g/100g
Dietary fibres	-	g/100g
Salt (NaCl equivalents)	1.8	g/100g

Stability of ketchups

The glass jars in which the ketchups and chutney were stored were placed in a dark cooling storage room for three weeks without being manipulated, then inspected to check the stability of the products within their containers.

It was noted that the ketchups showed a minor sedimentation effect with the creation of a thin film of translucent liquid at the top (Fig. 2.28). Under the effect of the maizena and pectin the sedimented mass retained its shape well and had a gelatinous-like appearance when gently turning the jar. However, the ketchup could easily be turned fully homogeneous again after a short period of shaking by hand. After shaking the ketchup regained the appearance it had when freshly prepared. This behaviour is not seen in commercial ketchups such as the reference sample, but in the commercial products additives are added to prevent sedimentation and retain a homogenous appearance, while the fresh preparations are additive-free, using only fresh products without the use of E-numbers or preservatives.

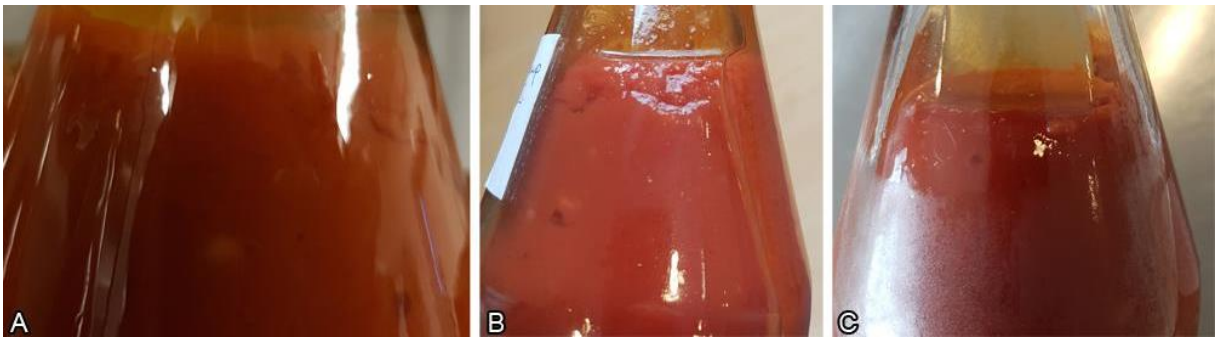

Fig. 2.28: Separation between liquid phase and solid phase in ketchups after three weeks of storage. A: jar bottle in upright position, B & C: bottle held under a 90° angle to show the amount of liquid and the solid mass forming a gelatinous-like solid mass that is easily disrupted when shaking by hand.

Water activity (Aw) and acidity (pH)

The water activity A_w of a food item is an assessment of the amount of available water in it and is expressed on a scale from zero to one. A low water activity (close to 0) means there is little water available. A high value (close to 1) means there's a lot of water available. Microorganisms usually require a high-water activity for proper growth. A food with a low water activity is therefore less prone to spoilage by microorganisms. Once the water activity is low enough, bacteria and moulds won't be able to grow in the food anymore. The ketchups have typically a very high-water activity value, above 0,95 as seen in table 2.11. Our in-house ketchup preparations have a higher A_w value than the commercial ketchups. Even the chutney has a higher A_w value than the commercial ketchup. Apart from water activity other parameters also influence the potential for growth of spoilage microorganisms, such as the addition of sugar and vinegar that influences the taste and lowers the acidity of the product.

Table 2.11: Water activity (Aw) and Acidity measurements for the ketchups and chutney. The pH value is a mean value from three independent measurements of the same sample.

Sample	A_w	pH
Chutney	0,9679	3,8
Tomato Ketchup - Balsamico : Maizena	0,9839	4,3
Tomato Ketchup - Balsamico : Maizena + Pectine	0,9832	4,1
Tomato Ketchup - Balsamico + Cherry : Maizena	0,9841	4,1
Tomato Ketchup - Balsamico + Cherry : Maizena + Pectine	0,9832	4,1
Paprika ketchup - Maizena	0,9778	4,2
Paprika ketchup - Maizena + Pectine	0,9771	4,1
Heinz Tomato Ketchup (Reference)	0,9584	3,6

A low pH-value (pH < 7) means that a food is acidic. Tomatoes are by nature already acidic. Vinegar is added during the preparation and has a pH-value far lower than the tomatoes, bringing the final pH-value of the ketchup down substantially. Although the exact value will depend on the brand and type of ketchup, we observed that the commercial reference ketchup has a lower acidity than our preparations. Only the chutney has an acidity close to the reference ketchup. At this pH-value a lot of microorganisms do not grow anymore, which improves the conservation time of the ketchups. Thanks to the addition of sugar the acidic aspect of the ketchup is less perceivable by the consumer.

Colour analysis

The colour measurements show that the ketchups with maizena and their counterparts with maizena and pectin have near identical colours. This is expected since the addition of pectin does not influence the product colour. The sweet bell pepper ketchup is dark red with a hue of brown while the tomato ketchup has a bright lively red colouration. The commercial reference sample is somewhat located in between. The differences in colour allow to easily distinguish the different products from each other. No food-grade dye was added to the preparations, the resulting colours were obtained only by the natural colouration of the

ingredients. The natural red colour of the ketchups comes from a molecule called lycopene, a bright red carotenoid hydrocarbon, which is naturally present in tomatoes. All the products that were created have a visually appealing colour (Fig. 2.27).

Table 2.12: Colour measurements in the CIELAB colour space for the ketchups and chutney with class II tomatoes and sweet bell peppers. Values represent the mean values for three measurements per sample.

Sample	Colour		
	L*	a*	b*
Chutney	32,96	28,95	38,3
Tomato Ketchup - Balsamico : Maizena	32,01	28,6	28,6
Tomato Ketchup - Balsamico : Maizena + Pectine	32.20	28.58	28.95
Tomato Ketchup - Balsamico + Cherry : Maizena	33.12	26.88	28.43
Tomato Ketchup - Balsamico + Cherry : Maizena + Pectine	33.00	27.04	28.48
Paprika ketchup - Maizena	24.06	22.85	25.72
Paprika ketchup - Maizena + Pectine	24.25	22.43	25.45
Heinz Tomato Ketchup (Reference)	25.95	31.57	27.40

Viscosity of tomato and sweet bell pepper ketchups

The rheology of the tomato ketchups and the sweet bell pepper ketchups was measured and compared to a commercial reference sample. The results showed excellent reproducibility amongst sub-samples, indicating a very homogenous viscosity amongst the different ketchups.

Of all tested samples the reference sample (= commercial tomato ketchup) had the highest viscosity, although at a shear rate below 100 $\dot{\gamma}$ (rotations per second) viscosity was almost identical to the tomato and sweet bell pepper ketchups that were stabilised with a mixture of maizena and pectin.

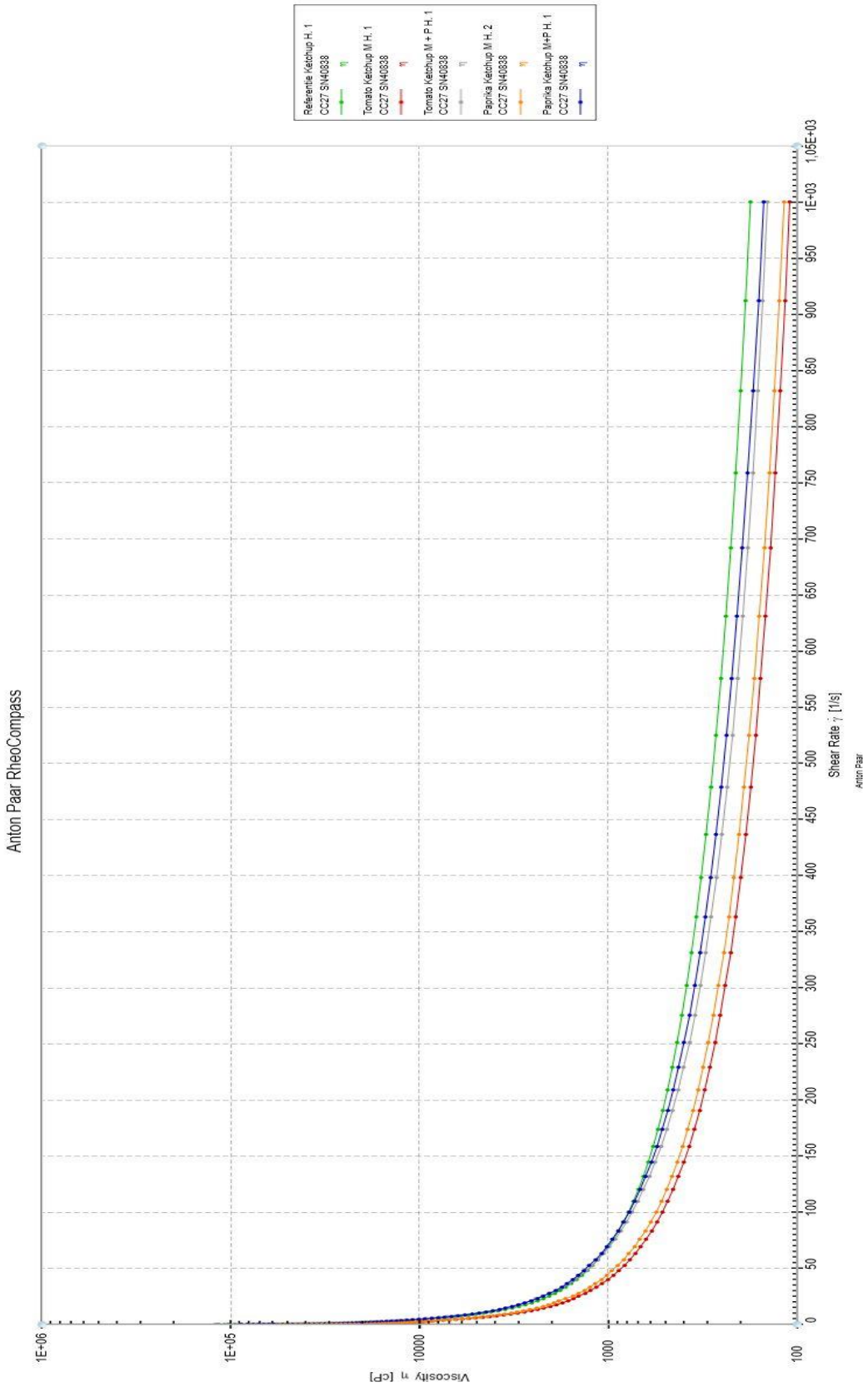

Fig. 2.29: Viscosity measurements for all sample types and a commercial reference sample. Viscosity ŋ expressed in centiPoise (cP) is plotted against shear rate γ̇, expressed in rotations per second (s^{-1}).

A small difference that very subtly builds up from that point onwards is detected with a difference of about 50 cP (centiPoise) at 1000 ẏ. The difference is much more perceptible when comparing the reference ketchup with our ketchups that were stabilised with only maizena. Here, the difference in viscosity is already detectable at much lower sheer rates. The ketchups with maizena and pectin are very close to the reference, while the variants with only maizena show a lower viscosity from very early on (Fig 2.29).

We can conclude that the use of maizena and pectin as binder for the sauce results in a viscosity that is much closer to the commercially available ketchups that the customer is familiar with, while the ketchups with only maizena tend to be slightly more liquid. Both variants are however identical in colour and taste. We conclude that the preparations with added pectin are better suited for commercial applications.

The tested ketchups show shear-thinning behaviour (= pseudoplastic flow behaviour) which is characterized by decreasing viscosity with increasing shear rates. Typical materials that also show this kind of behaviour are coatings, glues, shampoos, polymer solutions and polymer melts. Since viscosity is shear-dependent, it should always be given with the shear condition. Shear-thinning behaviour is related to the internal structures of the samples.

Conclusions and future perspectives

Pointed sweet bell peppers are a very versatile ingredient in many preparations and the creativity of the cook is often the only limitation to create new products or variations of existing products in which the peppers can be processed. This makes discard class II pointed sweet bell peppers an interesting by-product to be valorised. During this work done for the BioBoost project several producers of fruit-based products were showing their interest in the pointed bell peppers as an ingredient to create new lines of products, especially because of their taste and the natural deep red colouration that they add to the preparations. The company ‘Zoete Potjes’ showed a great interest to develop and add new products with class II pointed sweet bell peppers to their production line-up, which are already being sold in a wide range of stores, both local (artisanal resellers) and mainstream distribution (e.g. Delhaize – a well-known food and convenience orientated supermarket chain in Belgium).

Another company that develops ‘Fruit jellies (pâtes de fruits)’, here kept anonymous at the request of the company owner, also showed great interest to develop products that include sweet bell pepper by-products because of their unique colour and taste characteristics that can be added to the classic fruit jelly recipes.

In the course of this BioBoost project it became quickly clear that many future applications and opportunities for these by-products pointed bell peppers are waiting for the food processing industry to show interest and develop specific products based on them.

2.3.5. Fermented products

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

vives University of Applied Sciences KU Leuven Association

Introduction

In the report on the stabilization methods and techniques, fermentation was already investigated. This knowledge was used to develop some ready-to-eat products, based on 'misfit' vegetables. For this research two applications were developed: fermented tomato salsa and fermented pea-dip with mint.

Materials and methods

For the fermentation sterile jars with an airlock were used. Once the fermentation was finished, the airlock was removed, a normal lid was placed on the jar and the fermented products were stored in the refrigerator.

Fermented tomato salsa

All vegetables are washed and cut, the spices were added and everything was cut into a sauce. This sauce was cooked for 2 hours with open lid until the mixture had a good consistency. The salsa was transferred to a sterile jar with closed lid and cooled down. The starter culture (*Lactobacillus plantarum*) was added at room temperature and mixed with a sterile spoon. A lid with airlock was placed on the jar and the jar was kept at room temperature for at least 3 days. Every day the salsa was mixed with a sterile spoon and the pH was measured until pH was 3.6. Afterwards, the jar can be placed in the fridge with a closed lid or can be kept in a dark cool place with the airlock lid.

Fermented pea-dip with mint

Mint leaves are cut in very small pieces. The peas are mashed together with the salt and the mint and transferred to a big jar (fill maximum 75%), the jar is left for 10 minutes, this way, the liquid comes out of the peas. The peas are pressed down so that the liquid comes to the surface, a little more water is added when necessary to have enough brine. A bag filled with water is put on top of the peas to keep them submerged in the brine and the jar is covered with a cloth. The peas are fermented for 3 days at room temperature, after 3 days the brine is removed, fresh cream and pepper is added and everything is mixed with a blender to make a smooth dip.

Results

Fermented tomato salsa

The fermented tomato salsa is fresh sour and spicy. Due to the fermentation there is a little tingling mouthfeel. The salsa makes a perfect match with tortilla crisps but can also be used

in a pasta. This product is very accessible for people that want to become acquainted with fermented vegetables.

Table 2.13: Ingredient list for the fermented tomato salsa

FERMENTED TOMATO SALSA	
fresh tomatoes	85,00%
green celery	5,67%
cane sugar	4,53%
onion	3,97%
fresh garlic	0,57%
sea salt	0,08%
cinnamon	0,06%
cayenne pepper	0,06%
white pepper	0,06%
cloves	0,02%

Starter culture: *Lactobacillus plantarum*

Fig. 2.30: Top view of the finished product, a fermented tomato salsa.

Fermented pea-dip with mint

This pea-dip with mint is sour and a little salty, with a fresh twist of mint. The pea-dip is very nice with breadsticks, but can also be used as a spread on toast.

Table 2.14: Ingredient list for the fermented pea-dip with mint

FERMENTED PEA-DIP WITH MINT	
fresh or frozen peas	63,94%
fresh cream	31,97%
mint	2,56%
salt	1,28%
pepper	0,26%

2.3.6. Green Bean burger

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

vives University of Applied Sciences KU Leuven Association

Introduction

The aim of the development of the green bean burger was to make a vegetarian burger from residual flows from green beans in the freezing industry. These waste streams normally go to the digester to produce biogas. After selecting the right waste stream, a vegetable burger was developed using as much of the waste stream as possible.

Materials and methods

Selection of the waste stream

The company 'Ardo'[1], a company with expertise in fresh-frozen vegetables, herbs and fruit, in Ardooie was visited to map their processing loss, the loss obtained from the classification and cleaning step, during the production of frozen green beans. When the freshly harvested beans arrive at the company, first, the beans that are still clustered are separated from the single beans. These clusters make the first waste stream (fig. 2.31 left), the amount of this waste stream depends on the weather, when there was a very dry season there are more clusters. The second waste stream consists of the remaining stems, very thin beans and beans with discoloration (e.g. from spoilage). (fig 2.32) A third waste stream is created when cutting the beans in smaller pieces, everything that is too small, too big or has discoloration is removed before the beans go to the freezing tunnel. (fig 2.33)

Fig. 2.31: first waste stream (left after collecting, right after cleaning manually

[1] https://ardo.be/en

Fig 2.32: second waste stream

Fig. 2.33: third waste stream

The third waste stream is not fit for human consumption because of the possible presence of *Solanum nigrum* and *Datura stramonium*, both toxic for human.

The second waste stream has the advantage that the beans are already washed, but because of the high humidity and the presence of spoiled beans, the storage time of this waste stream is 1-2 days, which is too short. The amount of this waste streams is also not high enough to provide enough beans to use in a burger production.

The first waste stream, with the clustered beans, was selected to continue with because it produces the highest amount of usable beans, is easy to keep separate from the other waste streams and can be stored easily in the refrigerator for several days. The disadvantage is that the beans need to be separated manually before they can be used in the production of for example a burger (fig. 2.31 right).

Product development

An important aspect of a vegetarian burger is the protein content. Consumers choose a vegetarian burger tot substitute meat in their meal. Meat has a protein content of minimum 20%, whereas most vegetarian burgers have a protein content from 5 up to 10%. Therefore, it is of interest to reach a protein content as high as possible. Since vegetables are not an excellent source of proteins, it was important to add ingredients, which do contain a high amount of proteins. In this burger bulgur and nuts are the ingredients that add protein. Bulgur was selected due to its high content of carbohydrates and proteins. The carbohydrates are important to form a biopolymer network which can hold moisture, fat and flavours. In addition, bulgur has an adequate swelling capacity whereby it could easily absorb the excess of water of the green beans.

The bulgur was first boiled as prescribed on the package, then mixed with a blixer together with the olive oil and the soy sauce. Green beans were blanched and cuttered into small pieces, cashewnuts are also broken (not powdered so that they can give some structure in the burger), onion is cut into small pieces. All ingredients are mixed together and burgers are made of this paste. Burgers are baked in olive oil for 6 minutes, turning them regularly.

Results

The final composition of the green bean burger (fig. 2.34) can be found in the table below, as well as the protein content and the fiber content.

Table 2.15: the final composition of the green bean burger

Green bean burger	
Green beans	55,00%
Bulgur (boiled)	18,00%
Cashewnuts	15,00%
Onion	4,00%
Corn starch	3,00%
Bread crumbs	2,00%
Olive oil	1,20%
Soy sauce	0,80%
Nutmeg	0,50%
Salt	0,50%
Protein content	12,09 E%
Fibre content	6,70%

This vegetable burger is a 'source of protein', which means that at least 12% of the energy value of the burger is provided by protein. The green bean burger also meats the claim 'High in fiber', this claim can be made when a product contains at least 6 g of fiber per 100g. As the burger has less than 20% of protein it is not a full meat replacer.

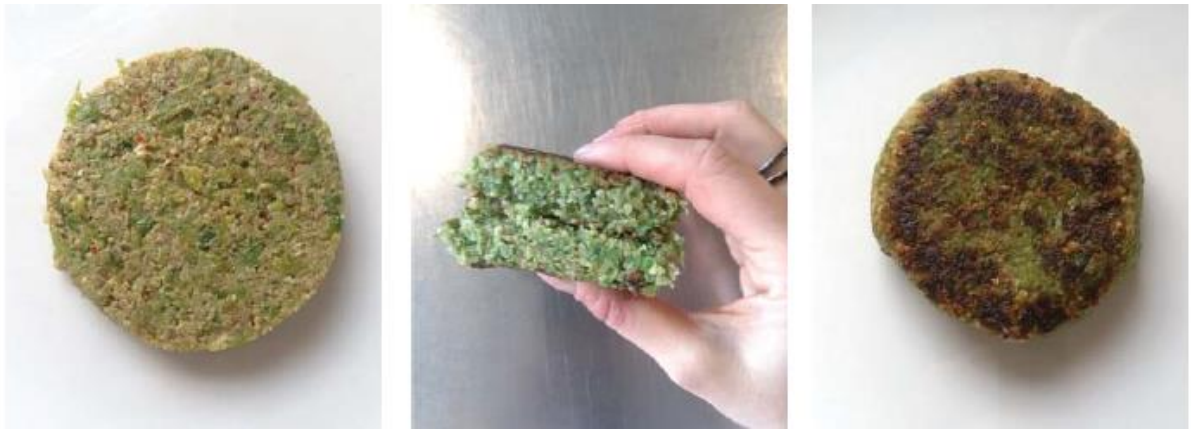

Fig. 2.34 Green bean burger. Raw (left), inside baked (middle), outside baked (right)

As green beans do not have a very pronounced taste, this green bean burger also has a rather neutral taste. Replacing part of the beans by other complementary vegetable residual flows can improve the taste. Therefor this burger can be seen as a base to make other vegetable burgers. The bulgur and the nuts give a nice firm structure to the burger with a good bite that is not too soft.

2.3.7. Spent grain burger with bell pepper

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

vives University of Applied Sciences KU Leuven Association

Introduction

Brewer's spent grain (BSG) is the residue left after separation of the wort during the brewing process. This BSG in often picked up by the farmers to feed directly to the cows. The composition and taste of BSG may vary with barley variety, time of harvest, characteristics of hops and brewery technology. Brewer's spent grains are of high nutritive value and contain cellulose, hemicelluloses, lignin and have a high protein content. Because of this high protein and high fiber content the BSG was selected to make a vegetarian burger, together with misfit bell pepper.

Materials and methods

Fresh spent grain was obtained from the brewery and dried at 105°C (hot air) until the BSG contained 30% dry matter, the drying time depends on the moisture content of the BSG.

The bulgur was first boiled as prescribed on the package, then mixed with a blixer (robot coup) together with olive oil and soy sauce to make a paste. Cashewnuts are broken in small fragments (<0.1cm), onion and bell pepper are cut into small pieces. All ingredients are mixed together and burgers are made of this paste.

The burgers are baked in olive oil during 8 minutes turning them regularly.

Results

Table 2.16: The final composition of the spent grain burger.

SPENT GRAIN BURGER	
BSG	36,00%
Bulgur	23,00%
Cashewnuts	10,00%
Bell pepper	20,00%
Onion	4,00%
Corn starch	2,00%
Cajunherbs	1,50%
Olive oil	1,50%
Mustard	1,00%
Soy sauce	1,00%
Protein content	16,54 E%
Fibre content	8,15%

This vegetable burger is a 'source of protein', which means that at least 12% of the energy value of the burger is provided by protein. The spent grain burger also meats the claim 'High in fiber', this claim can be made when a product contains at least 6g of fiber per 100g. As the burger has less than 20% of protein it is not a full meat replacer.

Fig. 2.35: Spent grain burger with bell pepper

The spent grain gives this vegetable burger a slightly bitter taste, the taste of the bell pepper is pronounced. The burger is easy to handle and has a good bite due to the vegetable pieces, nuts, bulgur and BSG. If the spent grain is too rough it is possible that the present fibres are a little bit disturbing, therefor not every BSG is equally suited to use without pretreatment.

2.3.8. Spent grain cookies

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

Introduction

Brewer's spent grain (BSG) is the residue left after separation of the wort during the brewing process. The taste of the BSG is very specific for each beer. A simple recipe was developed for the brewers to use part of this waste stream to make their own cookie with the specific taste of their beer.

Materials and methods

Brewers spent grain was obtained from the brewery and dried with hot air at 70°C for 10 hours until the BSG was dry enough to make a flour of it. The BSG was powdered and sieved (0.5mm).

The BSG flour was mixed with wheat flour and sugar, cold butter was added in small pieces and egg was added. Everything was mixed to make a firm dough. The dough was placed in the fridge to rest for 1 hour. The dough was rolled out until it had a thickness of 0.5cm and round shapes were pressed out.

The cookies were baked for 5 minutes in a waffle maker.

Results

Table 2.17: The composition of the cookies.

SPENT GRAIN COOKIES	
Sugar	34,00%
Spent grain flour	18,00%
Butter	17,00%
Whole egg	16,00%
Wheat flour	15,00%

Fig. 2.36: BSG cookies

Baking the spent grain cookies in a waffle maker gives them a nicer 'baked' taste then when they are baked in the oven (15 min 180°C). The cookies are rather hard, but crunchy. The taste resembles a sweet butter-cookie. The use of different spent grain will result in a different taste of the cookie which makes them unique for every brewer.

2.3.9. Yacon ice cream

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

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Introduction

Yacon is a root vegetable (Fig. 2.37) that originates from the Andes. The roots of the yacon contain a lot of fructo-oligosaccharides (FOS), that have a prebiotic function. Yacon can be eaten fresh, it has a typical slightly sweet taste, but it oxidates very fast once cut open. This way it is difficult to develop products with yacon that have a light color. For the moment, yacon is mainly used to make syrup from the juice (Fig. 2.38), the pulp (40% of the complete yacon) is normally not recovered. The pulp consists of 85% of moisture and 10% of fibers and has a sweet taste. The remaining 5% mainly consists of proteins and sugars. In this project the pulp is used to make ice cream that contains less sugar than the normal dietary ice cream, the yacon pulp is used, together with sucraless (a sweetener based on isomalt, sucralose and inuline with polydextrose as filler) to make a tasty ice cream with the specific taste of yacon.

Fig. 2.37 :Yacon root

Fig. 2.38: yacon syrup

Materials and methods

Yaconpulp

In order to obtain yacon pulp, yacon was peeled and immediately submerged in cold tapwater to avoid further color changing. Peeled yacon roots were mixed in a blixer together with 0.25% of ascorbic acid in order to prevent oxidation. This puree was transferred into the juicer where the pulp and the juice were separated. The obtained pulp was pasteurized at 65°C during 1 minute, cooled down and stored in the freezer.

Preparation of the ice cream mix

Wet ingredients are weighed and placed in the blender, the blender is set on low speed and slowly the dry ingredients are added, when this is mixed well, the blender is set on high speed

and everything is mixed during 1min/L. This mix is then pasteurized in the ice cream machine, 10 sec at 45°C and 1 minute at 85°C.

After pasteurization, the viscosity is checked with the viscosity meter (Brookfield DV2Textra), using spindle 04, the viscosity before aging should be in between 300-400 mPa.s

The mix is stored in a sealed bag and placed in the fridge of at least 4h to let it ripen.

Ice cream making

The aged mix is poured in the blender on low speed and 5g of ice cream softener (Softin from MEC3: stabilizer: sorbitol syrup, emulsifier: mono- en diglycerides, water, flavouring) is added, the blender is set at high speed during 1min/L. Transfer the content into the ice cream machine (Fig. C) and start the machine. Leave the ice cream at least 12h in the freezer to harden.

Fig. 2.39: ice cream machine (Valmar)

Fig. 2.40: yacon ice cream

Results

There were different challenges when making ice cream with yacon pulp. First, because of the oxidation the color of the ice cream became too dark in comparison with a standard white vanilla ice, which made it less attractive (Fig. 2.40). When adding ascorbic acid before the juicing process, the oxidation was prevented and the color of the ice cream was acceptable.

As the aim was to make an ice cream with no added sugar a second challenge was to make the yacon ice cream to be sweet enough, but not too sweet, no after taste because of the use of sweeteners was wanted and the taste of the yacon needed to be present. Sucraless was selected as the best sweetener in this product.

Fig. 2.41: yacon ice cream without ascorbic acid

The scoopability of the ice cream is also a very important parameter when developing an ice cream. The scoopability depends on the lowering of the freezing point due to the presence of sugar. As the aim was to add no sugar in the yacon ice this was a third challenge. The ice cream softener 'Softin' was added to improve the structure of the ice cream.

Table 2.17: The final recipe for the Yacon ice-cream

YACON ICE	
Whole milk	50,00%
Yacon pulp	23,00%
Sucraless	15,00%
Cream 40%	6,00%
Softin	5,00%
K-carrageenan	2,00%
Guar gum	1,00%

Table 2.18: Nutritional values for the Yacon ice-cream. (*) mean values from 'www.voedingswaardetabel.nl'

Nutritional value /100g			
	standard ice cream(*)	**yacon ice**	**% reduction**
energy (kcal)	257	97,4	62%
protein	3,9	3,13	
carbohydrates	23,5	11,22	52%
sugars	20	3,52	82%
fat	16,5	4,44	73%
fibers	0,1	2,3	

When comparing this yacon ice with standard ice (vanilla ice without coloring), we can say that yacon ice contains 62% less calories, 82% less sugar and 73% less fat and is suited for diabetic.

This yacon ice cream is not very pronounced in taste, so it can be used as a base for other ice-cream tastes. A vanilla and chocolate variant were made, and as some people also found that the yacon taste in the base was not clear enough, yacon syrup was added to the base to make an ice-cream with the real yacon taste (Fig. 2.42)

Fig. 2.42: variants of yacon ice cream: left: vanilla, middle: yacon (base + yacon syrup), right: chocolate

2.3.10. Crispy horse cookie made of spent grain and apple pulp Introduction

Developed at VIVES Hogeschool

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vives University of Applied Sciences KU Leuven Association

Introduction

Brewer's spent grain (BSG) is the residue left after separation of the wort during the brewing process. This BSG in often picked up by the farmers to feed directly to the cows. The composition and taste of BSG may vary with barley variety, time of harvest, characteristics of hops and brewery technology. Brewer's spent grains are of high nutritive value and contain cellulose, hemicelluloses, lignin and have a high protein content. Horse treats often contain certain quantities of grain, grain is known to be difficult to digest by a horse' intestinal flora but spent grain is 'predigested' and contain a lot of crude fibers which are crucial in a horse's diet.

The apple pulp that is used to make this treat tasty for the horse is a waste stream from the juice industry.

Materials and methods

Linseed is used as a binder, therefor it is first boiled with water until it becomes coherent.

The boiled linseed, apple pulp and spent grain are mixed together, the dough is rolled out with a thickness of 1,5cm and cookies are formed. The cookies are placed in the oven at 150°C for 4 hours. The water activity of the cookies is 0,34 on average, so micro-organisms cannot grow if the treats are packed in a packaged that prevents them from taking up moisture.

Fig. 2.43: Crispy horse cookie made of spent grain and apple pulp

Results

Table 2.19: The composition of the horse cookies.

CRISPY HORSE COOKIE	
BSG (fresh)	53,60%
Apple pulp	42,10%
Linseed (cooked)	4,20%

2.3.11. Fresh vegetable cheese

Developed at VIVES Hogeschool

Vanherpe, I., Rigole, F., Callens, A.

Introduction

Vegetables like butternut or carrot that are too big, too small or don't have the right shape often stay on the field. After harvesting the first bud of the main head of a broccoli plant, the plant produces smaller side shoots, these side shoots are not harvested. With these left over's a smooth fresh vegetable cheese was developed.

Materials and methods

Vegetables are cleaned and cut and are cooked in the steamer. The steamed vegetables are homogenized with a blender. After cooling down, herbs and fresh cheese are added and mixed with the vegetable puree to get a smooth structure.

Results

Fig. 2.44: The final preparation of butternut-carrot cheese and broccoli-celery cheese.

Table 2.20: Butternut-carrot cheese

BUTTERNUT-CARROT CHEESE	
Fresh cheese	49,3%
Butternut	37,5%
Carrot	12,5%
Chives	0,33%
Pepper	0,16%
Herbamare® original herbed sea salt	0,16%

Table 2.21: Broccoli-celery cheese

BROCCOLI-CELERY CHEESE	
Fresh cheese	49,7%
Celery	30,0%
Broccoli	20,0%
Pepper	0,16%
Herbamare® original herbed sea salt	0,16%

2.4. Discussion, recommendations and conclusions

Value-added products can be defined in various ways, but generally speaking they represent products that have an intrinsic added value over their more mainstream counterparts because of the way they are being produced, harvested, handled and/or processed into high quality foods. The added value can be an improvement of sustainability throughout the production chain from growing the crop to the preparation of health promoting niche products. In our case we focus mainly on the added-value by using by-products and food-waste fractions that would otherwise be lost. Therefore, there is an added economic value, by creating more sellable products for the same amount of raw material, thus generating a higher revenue for the growers and actors throughout the value chain. On the other hand, there is value that is being generated in terms of sustainability and efficiency. By reducing the waste streams, a more durable, sustainable and less environmentally harmful production cycle is adopted, which reduces the costs for the handling and disposal of the waste, and simultaneously reduces the stress on the environment that waste streams can inflict. Some unused crops or by-products from agriculture and horticulture are returned to the land when left unused, thus charging the land with an excess of nutrients and plant material that could be harmful for the environment.

An important way to reduce waste fractions from the production of food is to use second grade (class II) fruits and vegetables who have been discarded at the auctions during the selection for the fresh market. These class II products are often equal in quality as the high-grade selected ones that are destined for the fresh market, however, due to arbitrary quality parameters such as appearance, colour or size, these products cannot be sold on the fresh market and form a waste fraction. Some of the Class II fruits and vegetables can still be marketed by discount retailers, but the remaining fraction is simply treated as waste and discarded from the food chain. Creating processed foods based on these class II discards can lead to semi-processed or fully processed ingredients for the preparation of convenience foods of equal quality as the high-graded first choice ingredients, they also add value by reducing the amount of waste fraction that is generated during the production chain.

Value-added foods have the advantage that they can open new markets, enhance the public's appreciation for particular products, and/or extend the marketing potential of the source materials that are being used. Additionally, the creation of novel applications, tailored specifically for the by-products that are valorised, can lead to the development of completely new processed products that were not previously not available. For example, within this project we investigated the potential use of cooking fluids from legumes for the preparation of vegetarian and vegan meals, which are highly sought after by a growing number of food-conscient people who actively explore the possibilities to replace some traditional animal-based ingredient by equivalent plant-based alternatives.

However, to become a success-story the valorisation of by-products as a novel processing strategy and market application needs to establish a balance that leads to a win-win scenario for all involved parties. This means that a strong marketing strategy needs to be developed to stimulate the introduction of new products and product lines on an already highly saturated market in order to provide a strong appeal for potential customers, which is often difficult to realise for small players. Large resellers and food-store chains are better suited to reach out to the customers and to stimulate the attractiveness of these products towards a larger public. Collaborations between growers, processors and retailers/resellers/distributors are key to give by-product based value-added foods the momentum that they need to make the use of by-products attractive and profitable. A few of these collaborations already exist and could stimulate others to follow. One example given here is the Colruyt group[1] in Belgium that manages several supermarket chains including the Colruyt stores (food and non-food) and Bio-Planet stores (bio-based food) who specialise in sustainable bio- and ecological foods. Colruyt already works together with growers and processors and promotes novel products from smaller parties that would otherwise not have the possibility to appeal to a broader audience.

The collaboration between processors and sheltered workshops that can produce processed foods on demand is also very important to allow for scalability in the production of by-product-based foods. Sheltered workshops can be very versatile and provide essential logistic support for storage of fresh products, specific processing steps such as washing and cutting or even upscale the production by taking over processing and preparation processes as a whole. The value of these sheltered workshops lies in their versatility, flexibility, scalability and the outstanding quality of the foods that they prepare. One out of many examples is the company EnVie that produces soups from fresh surplus vegetables supplied by Belgian farmers. Their activities currently allow them to save up to 50 tons of surplus vegetables per year. The soups are produced by a team of passionate people who find employment thanks to EnVie after a period of long-term unemployment. The Reo auction[2] is an important partner of EnVie and supplies the surplus of fresh vegetables directly to them. These vegetables are extremely suitable for making soup (zucchini, leek, celery, tomato, etc.). The soups that EnVie makes are on their turn distributed by the Colruyt Group via their network of stores and resellers, closing the cycle to maximize efficiency and outreach for the high-quality value-added products that have been produced using by-products.

[1] https://www.colruytgroup.com/wps/portal/cg/nl/home/pers/press-releases/Sociale+onderneming+enVie+stelt +langdurig+werklozen+te+werk+om+verse+soepen+te+produceren+uit+groentesurplus

[2] http://www.reo-veiling.be/nl/nieuws/partnership-met-envie-24

3. Conservation techniques

3.1. Introduction

Conservation techniques aim to prolong the shelf life of food. This is the maximum time that a food item (processed or unprocessed) can retain all of its organoleptic, nutritional and health properties. Various microorganisms and fungi are naturally present in the environment and can easily contaminate food by contact with air, or during the various handling and preparation processes. When conditions are good their growth will inevitably result in the spoilage of the food, thus causing it to become unpalatable or making it unsafe for consumption.

A variety of techniques can be applied to boost the conservation period and prevent the development of spoilage inducing organisms. The most common conservation techniques are listed here:

- **Cooling**: Either at low temperatures above the freezing point, then we talk about refrigeration (Between 0°C and 5°C), or at temperatures below the freezing point, then we talk about freezing (less than -18°C). Depending on the matrix refrigeration allows to extend the shelf life with many days (sometimes weeks), while freezing can allow for conservation spanning over many months.
- **Heating**: Microorganisms that are responsible for spoilage are destroyed by a heat treatment. The most popular methods are: pasteurization, cooking, sterilization and ultra-pasteurization.
- **Drying**: Removing a portion of the water contained within the food item prevents microorganisms from developing by using the moist naturally present in the food matrix to grow out and form colonies. Removing water can be achieved by drying (usually by adding heat to speed up the evaporation process), by salting, sugaring, or smoking.
- **Adding preservatives**: Some compounds can be added to food to stop or mitigate the growth of undesired micro-organisms. For example, preservatives such as Benzoic acid (E210) or lactic acid (E270) can alter the acidity by lowering the pH and block the development of microbes.
- **Packing under modified atmosphere**: Micro-organisms need oxygen to develop, by removing the air within the package and replacing it with a mixture of other gases such as CO2 (Carbon dioxide) and N2 (Nitrogen gas) the development of the microorganisms is greatly reduced or prevented.
- **Canning**: Canned food is sealed in an atmosphere free container, then heated to a target temperature for a short time and quickly cooled down. The heat kills off any spoilage inducing micro-organisms, while the atmosphere-less environment prevents

the growth of any potentially remaining germ. When food is canned there is no need to add any preservatives.

- **Fermentation:** In this chapter on value-added products we also explored the use of fermented products as ingredients for the preparation of meals. Fermentation is a natural process that alters the physico-chemical characteristics of food. It is the conversion of sugars and other carbohydrates into alcohol or preservative organic acids and carbon dioxide.

The duration of the conservation period of food items can be determined or monitored by performing microbiological essays and checking the bacterial growth by inoculating a sample onto a growth medium in a petri dish to detect the amount of colony forming units (CFU) that were originally present in the sample.

3.2. Aim

The aim of this chapter is to assess the conservation potential for a selection of our product innovations in which we used by-products of fruits or vegetables.

3.3. Case studies

3.3.1. Shelf-life stability of pasteurised tomato Juices

Developed at ILVO in partnership with 'Tomabel'[1]

Van Droogenbroeck, B., Vermeersch, X., Vlaemynck, G.

ILVO
Flanders research institute for agriculture, fisheries and food

Introduction

The tomato juice recipes that were developed as value-added products and discussed in their respective chapter earlier in this report were conserved under controlled conditions for a target period of one year (12 months) to check for conservation shelf life and verify potential post-contamination during packing and conservation.

Methods

The three tomato juices that were developed in a collaboration between Tomabel and ILVO (discussed in more detail in the Value-added chapter of this report) were stored at two different temperatures, room temperature (21°C) and refrigerator temperature (7°C) and tested for microbiological contamination at the start date, after two weeks, after one month, after six weeks, two months, six months, eight months, ten months and one year.

Additionally, the odour and colour were assessed using the in-house method developed at ILVO to verify the olfactory and visual appeal of the conserved juices.

[1] http://www.tomabel.be/

Results

Table 3.1: Microbiological analysis of the three tomato juice recipes at 21°C

21°C - Tomato-Basil juice											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (30°C)	cfu/ml	ISO15214	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,47	4,35	4,45	4,41	4,47	4,43	4,35	4,36	4,23
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moults (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Aerobic bacteria (30°C)	cfu/ml	ISO 4833 (part 1)	$2,7.10^{1}$	$9,1.10^{0}$	$9,1.10^{0}$	$1,8.10^{1}$	$1,8.10^{1}$	<10	$9,1.10^{0}$	9,1.100	$1,8.10^{1}$

21°C - Spicy Tomato juice											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (30°C)	cfu/ml	ISO15214	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,6	4,38	4,44	4,43	4,51	4,44	4,38	4,34	4,25
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	< 10	<10	<10	<10	<10	<10	<10	<10
Moults (25°C)	cfu/ml	ISO 7954	< 10	< 10	<10	9,1.10^0	<10	<10	<10	<10	$9,1.10^{0}$
Aerobic bacteria (30°C)	cfu/ml	ISO 4833 (part 1)	$1,8.10^{1}$	$1,8.10^{2}$	<10	< 10	$9,1.10^{0}$	<10	<10	<10	$1,8.10^{1}$

21°C - Tomato-Celery											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (30°C)	cfu/ml	ISO15214	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,17	4,36	4,45	4,43	4,46	4,44	4,4	4,36	4,23
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moults (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Aerobic bacteria (30°C)	cfu/ml	ISO 4833 (part 1)	$1,0.10^{2}$	$5,5.10^{1}$	$1,8.10^{1}$	$3,6.10^{1}$	$9,1.10^{0}$	$9,1.10^{0}$	<10	<10	<10

Table 3.2: Microbiological analysis of the three tomato juice recipes at 7°C

7°C - Tomato-Basil juice											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (44°C)	cfu/ml	ILVO method	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,47	4,35	4,42	4,42	4,45	4,44	4,4	4,35	4,25
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	$9,2.10^{2}$	< 10	< 10	< 10	<10	<10	<10	<10
Moults (25°C)	cfu/ml	ISO 7954	2,7.10^1	< 10	< 10	$5,5.10^{1}$	< 10	<10	<10	<10	<10
Aerobic bacteria (21°C)	cfu/ml	ILVO method	< 10	$1,0.10^{3}$	$6,4.10^{1}$	$2,7.10^{1}$	$9,1.10^{0}$	<10	$6,4.10^{1}$	$5,5.10^{1}$	$9,1.10^{0}$

7°C - Spicy Tomato juice											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (44°C)	cfu/ml	ILVO method	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,6	4,38	4,41	4,43	4,49	4,45	4,42	4,34	4,27
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	<10	<10	<10	<10	<10	<10
Moults (25°C)	cfu/ml	ISO 7954	$1,8.10^{1}$	< 10	< 10	$2,7.10^{1}$	<10	<10	<10	<10	<10
Aerobic bacteria (21°C)	cfu/ml	ILVO method	< 10	$9,1.10^{0}$	$5,5.10^{1}$	<10	$3,6.10^{1}$	<10	$1,8.10^{1}$	$1,8.10^{1}$	$1,8.10^{1}$

7°C - Tomato-Celery											
Analysis	**Unit**	**Method**	**T0**	**2 Weeks**	**1 Month**	**6 Weeks**	**2 Months**	**6 Months**	**8 Months**	**10 months**	**12 months**
Lactic acid bacteria (44°C)	cfu/ml	ILVO method	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Acidity (pH)	-	-	4,17	4,39	4,4	4,45	4,47	4,45	4,43	4,02	3,97
Yeasts (25°C)	cfu/ml	ISO 7954	< 10	< 10	< 10	<10	<10	<10	<10	< 10	< 10
Moults (25°C)	cfu/ml	ISO 7954	$1,0.10^{2}$	< 10	< 10	$6,4.10^{1}$	<10	<10	$9,1.10^{0}$	< 10	< 10
Aerobic bacteria (21°C)	cfu/ml	ILVO method	< 10	$9,1.10^{0}$	$1,9.10^{2}$	$1,2.10^{2}$	$4,6.10^{1}$	$8,2.10^{1}$	$1,1.10^{2}$	$2,6.10^{2}$	$3,6.10^{1}$

Table 3.3: Microbiological guidelines as taken from: 'Microbiological guidelines: support for interpretation of microbiological test results of foods. Elaborated by the Food Microbiology and Food Preservation Group (FMFP-UGent). ISBN 9782874035036'. Threshold limits are expressed in colony forming units per millilitre (cfu/ml). These reference values apply for pasteurised juices of vegetal origin Cat 4I.

Analysis	Unit	Target	Tolerance	Use by date
Lactic acid bacteria	cfu/ml	3.10^1	3.10^2	3.10^2
Aerobic bacteria	cfu/ml	3.10^3	3.10^4	3.10^6

The results of the microbiological assessment shows that after twelve months of conservation all values remain well below the target values for Cat 4I pasteurised juices of vegetal origin as indicated in table 3.3. This indicated that the pasteurisation process was successful at keeping the juices safe for consumption after an extensive period of time. Colour and odour were judged satisfactory and unaltered during the entire conservation period for all samples. The low acidity of the juices, ranging between 3.97 and 4.6, is an additional growth inhibitor that helps to maintain a long shelf-life for the tomato juices.

3.3.2. Conservation of fresh ravioli with a filling of class II Belgian endives

Developed at ILVO in partnership with the food company "PASTATI"[1]

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Introduction

Belgian endives, as said before, are known as the Belgian 'white gold' by the local growers. Although the Belgian endive roots are the most important by-products from the production, the outer leaves that are removed before final selection of the Belgian endives can also be used. While the outer leaves have a good nutritional value, these are seen as a waste fraction and currently not used in further processing and valorisation.

This chapter discusses in more detail the conservation potential of the fresh ravioli with Belgian endive filling that was discussed in more detail in the value-added chapter of this report.

Methods

A semi structured interview was conducted with the owner of Pastati and the challenges in the field were discussed. Pastati is a local enterprise that balances corporate social responsibility (CSR) with the empowerment of the local economy. They make a variation of fresh (uncooked) pastas, e.g. raviolis with a vegetable stuffing. Pastati also embraces a sustainable and eco-friendly approach in the selection of ingredients and packaging of their products. The vegetables need to be local, organic and fresh and no chemicals or preservatives are added during the production process. There is a close collaboration with a sheltered workshop to scale up the production of the finished product and for the storage of ingredients and pre-processing of ingredient batches such as cleaning and cutting. This collaboration allows us to better suit the needs of the clients and quickly adapt the scale of production accordingly. The use of fresh products and short storage times is ideal for a product on demand approach.

For the stuffing of the ravioli, Belgian endives (whole endive heads) of category II were used. There is also a strong interest to use the discarded outer leaves of the freshly harvested Belgian endive crops if the use of class II Belgian endives turns out to be a success. The freshly prepared pasta was delivered at ILVO where it was immediately processed for microbiological analysis. A portion of the ravioli's were also sealed under MAP conditions (modified atmosphere packaging, with a 70/30 mixture of nitrogen and carbon dioxide gas)

[1] https://www.pastati.be/

Results

Challenges in the real world

The stream of by-products, used for the stuffing, is dependent on the offer of the farmers. Therefore, a flexible and quick anticipation needs to be made on both sides. The basic rules of supply and demand need to be taken into account, because the price of the by-products is always fluctuating. And most farmers take advantage of the demand and raise their price for the by-products.

Secondly, the washing-step of the vegetables is very important, but also brings different problems. At this moment, it's impossible to outsource this step. Although it's very important prior to the preparation in the sheltered workshop.
A ravioli filling was made using second class Belgian endives, more information on the development of this product is available in the "value added" chapter of this report.

Microbiological assessment and conservation tests

After the preparation of the ravioli, the microbiology of the product was assessed using seven different parameters. A part of the product was packed in a modified atmosphere (70/30) at 7°C. To slow down the microbiological growth. The other part was packaged in the open air and also stored at 7°C. First on day 0, then after three days the same parameters were analysed.

This product can be categorized in the group of the REPFEDs (refrigerated processed foods of extended durability), who are susceptible to post-contamination. This group includes cooked chilled foods that are portioned after heat treatment. This food can be used in B2B settings. The shelf life of these cooked and chilled foods is normally several weeks.

Table 3.4: Overview of the microbial counts for the Belgian endive filled ravioli for the two packaging methods

Packaging with product exposed to air

Parameters		Day 0	Day 3	Day 5	Day 7
Preservation	Total Aerobic psychrotrophes (cfu/g)	$1{,}7.10^5$	$6{,}7.10^6$	$1{,}7.10^8$	$2{,}0.10^9$
	Total Anaerobic psychrotrophes (cfu/g)	$1{,}0.10^5$	$7{,}6.10^5$	$1{,}2.10^8$	$2{,}0.10^8$
	Psychrotrophic Lactic Acid Bacteria (cfu/g)	$9{,}7.10^4$	$7{,}4.10^5$	$1{,}2.10^7$	$1{,}9.10^8$
	Yeasts (cfu/g)	$6{,}8.10^4$	$4{,}0.10^5$	$7{,}0.10^5$	$1{,}5.10^6$
	Moults (cfu/g)	< 10	< 10	<100	<100
	Odour	ok	ok	ok	ok
	General Appearence	ok	ok but with dry aspect	ok, not completely dry anymore	ok, somewhat less dry than previously
Hygiene	E. coli (cfu/g)	< 10 cfu/g	-	-	-

MAP packaging (modified atmosphere 70/30)

Parameters		Day 0	Day 3	Day 5	Day 7
Preservation	Total Aerobic psychrotrophes (cfu/g)	$1{,}7.10^5$	$6{,}5.10^5$	$1{,}3.10^7$	$7{,}9.10^7$
	Total Anaerobic psychrotrophes (cfu/g)	$1{,}0.10^5$	$3{,}2.10^5$	$8{,}1.10^6$	$9{,}2.10^7$
	Psychrotrophic Lactic Acid Bacteria (cfu/g)	$9{,}7.10^4$	$4{,}2.10^5$	$8{,}9.10^6$	$6{,}2.10^7$
	Yeasts (cfu/g)	$6{,}8.10^4$	$6{,}5.10^4$	$1{,}0.10^5$	$2{,}7.10^5$
	Moults (cfu/g)	< 10	$1{,}1.10^2$	<100	<100
	Odour	ok	ok	ok	ok
	General Appearence	ok	ok but the dough looks more sticky and gummy-like	ok, but sticky	ok, but sticky
Hygiene	E. coli (cfu/g)	< 10	-	-	-

Table 3.5: Microbiological guidelines as taken from: 'Microbiological guidelines: support for interpretation of microbiological test results of foods. Elaborated by the Food Microbiology and Food Preservation Group (FMFP-UGent). ISBN 9782874035036'. Threshold limits are expressed in colony forming units per gram (cfu/g).

	According to Category 6A guidelines for REPFED		
	Target	**Tolerance**	**Use by date**
Total Aerobic psychrotrophes (cfu/g)	$3,0.10^3$	$3,0.10^4$	$3,0.10^6$
Total Anaerobic psychrotrophes (cfu/g)	$3,0.10^3$	$3,0.10^4$	$3,0.10^6$
Psychrotrophic Lactic Acid Bacteria (cfu/g)	$3,0.10^2$	$3,0.10^3$	$3,0.10^7$
Yeasts (cfu/g)	$3,0.10^2$	$3,0.10^3$	$3,0.10^5$
Moults (cfu/g)	$3,0.10^2$	$3,0.10^3$	No visible moults
E. coli (cfu/g)	< 10	-	-

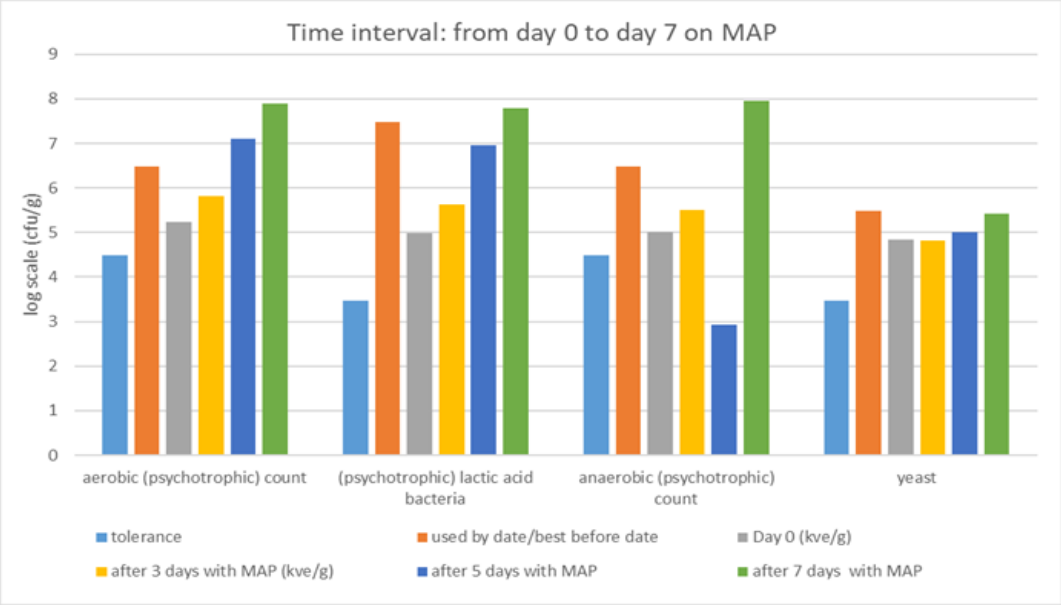

Fig. 3.1: Graphic overview of bacterial load on the fresh raviolis with Belgian endive filling, followed during 7 days under atmospheric packaging and MAP packaging.

3.4. Discussion, recommendations and conclusions

The microbial analysis of the fresh ravioli pasta with a filling of Belgian endives has revealed that the bacterial load is already very high at the time of production (day 0), and exceeds the recommended values after day 3. At day 5 the recommended values are exceeded and human consumption is no longer recommended. Furthermore, the storage of the fresh pasta also has a visible effect on the perception of the pasta. The fresh uncooked pasta is usually stored in a food grade plastic box, with a lid cover and on a piece of semi-permeable paper. Another sheet of paper covers the pasta.

The product remains exposed to the atmosphere since it is not hermetically sealed. Prior to storage, a small amount of flour is scattered on top of the fresh pasta to prevent sticking. When stored like this for a few days the outside layer of the ravioli tends to dry out a bit at

first, but the high moisture content filling inside causes the pasta to become somewhat moist after a while.

Alternatively, the packaging under MAP seals the product hermetically, leaving no chance for moisture to escape. It was observed that the fresh pasta quickly became sticky and had an unattractive moisty appearance. The MAP packaging was deemed unfit because it strongly altered the visual appearance of the fresh product. Because of the high initial microbiological load MAP packaging does not provide a significant improvement over atmospheric packaging to extend shelf life.

To improve the conservation potential of the fresh ravioli, an improved preparation process for the filling is proposed with higher heating temperatures to ensure a lower microbial count. Heating up the filling at a higher temperature for a longer period of time is necessary to achieve this. For this preparation the filling was heated up to 80°C for a very short period of time (about ten seconds) to avoid altering the organoleptic properties of the Belgian endive mix. This brief heating turns out to be insufficient to reduce the microbial load in the ravioli filling.

Additionally, a suitable packaging method is required. The company Pastati wishes to use eco-friendly packaging that allows recycling and has a small ecological footprint and is currently doing tests with packaging on sugar-cane fibre basis. At the time of writing no satisfactory solution is available, but future investigations in this matter will likely yield a suitable packaging method that allows for excess moist to be expelled, while shielding off the product from atmospheric contact.

4. General discussion, recommendations and conclusions

4.1. Challenges, bottlenecks and opportunities for the valorisation of by-products and food-waste fractions.

Using by-products and food waste fractions to create new products for market applications is generally speaking more challenging than to use mainstream fresh market products. This is due to variables that are less impactful in the large-scale fresh market production, but can become a limitation when focusing solely the use of by-products.

Here are some of the most common challenges and bottlenecks regarding the use of by-products and food waste fractions:

- **Availability and scale**: One of the main constraints in the development of food grade applications for by-products and food waste fractions is their availability. This is particularly important for continuous processes that require a constant supply of by-products. Availability can be influenced by seasonality of the source material and the number of by-products that are generated for each product. For example, Belgian endive roots are available all year round because of the long-term storage of roots that are being forced to produce Belgian endives all year round. There is always a substantial number of roots available since the number of roots is running in parallel with the total production of Belgian endives. But class II discards of Belgian endive heads that are not being sold on the fresh market are available in much lower quantities and depend on the quality of the produce itself. There might be strong fluctuations in their amounts throughout the year. Other crops such as seasonal fruits and some leaf vegetables that are only grown seasonally will only generate by-products during specific periods, which also strongly impacts their availability for processing. In order to be profitable, not only does the by-products need to match with certain quality standards, they must also be available in large enough amounts and during a long enough period throughout the year to avoid a production hiatus. Small and local processors are therefore more suited for working with by-products because of their versatility in how they choose the products they work with and their flexibility in regard to the amounts that are being processed. Larger players require a guarantee for more steady supply for industrial or semi-industrial production.
- **Intrinsic quality of by-products**: By-products and food waste fractions that are being valorised for the creation of food-grade applications result from discards in the selection and commercialisation process, or are the result of waste streams that are being created during the cultivation and harvesting process. Nevertheless, they must also obey very strict quality parameters in order to be safe for processing. It is

therefore very important to characterise each by-product to avoid any undesired compounds. One example are apple seeds in apple press cake that can contain elevated levels of cyanide and might be toxic if used for processed products. Despite being discarded from the selection for the fresh market, many class II products are perfectly safe to use, but apart from a deviating shape or colour, there must also be sufficient attention to avoid contamination with other product wastes or undesired components such as soil, detritus or other unwanted contaminants.

- **Respect of the cold-chain**: By-products that belong to class II discards have been processed in a similar way as the class I products prior to their by-product designation and therefore followed the cold chain and other strict regulations that fresh products are subjected to, to keep them safe for human consumption. To keep them food-safe and maximise their quality it is crucial that the cold-chain for by-products is respected all the way up to consumption in the same manner as is done for fresh market products. By-products that are generated earlier in the production such as by-products from harvest and from in-the-field processing need the same level of high-quality processing and conditioning. This new mindset of managing by-products as full equivalents to fresh produce is very important and needs to be applied thoroughly but by the producers and growers, and the companies and processors that utilise them. By-products are valuable products that would go to waste if not properly processed and utilised and are not a disposable waste stream sensu stricto.
- **Price:** An important incentive to work with by-products and food waste fractions as a primal resource for the preparation of processed food is their 'strongly' diminished price in comparison to the same product if it were sold as a fresh market graded product. There is however a danger that the demand for by-products grows beyond what is available and forces the prices to enter in competition with the originally sought-after fresh market product. In some cases, the by-product that was priorly regarded as a waste fraction could become a more valued product than what it was derived from. A good example of this is found in the dairy industry with milk whey. Whey is the liquid remaining after milk has been curdled and strained. It is a by-product of the manufacture of cheese or casein and although in the beginning it was merely a waste stream fraction, it now has several high-value commercial uses.
- **By-product specificity and opportunities for a biomass hub:** Although by-products are interesting to valorise, they are often very specific by nature and require also very specific pre-processing techniques and adapted processing lines to deal with them effectively and guarantee top quality processing for food applications. It is therefore very difficult to develop a general application that can deal with many different types of by-products. The diversity of by-products and waste fractions is as great as the

produce and production processes they are derived from. A case by case approach is often necessary to develop success stories.

- **Opportunities for a biomass hub:** Working with by-products poses many difficulties and challenges, mostly linked to the unpredictable availability in time (seasonality of products) and amounts (how much is available at a given time). The creation of a processing hub with multi-purpose equipment could be a strong promoter to both upscale the production and respond quickly to the unpredictable nature of the by-products themselves. A good example of multi-purpose equipment is the VaculIQ® press that is extremely versatile in terms of processing by-product streams. It can be used to press juices but also to extract press cakes and fibre rich fractions from practically any source material. The vacuum pressing additionally allows to obtain the highest quality oxidation free products that can either be further processed or used directly as a finished product.
- **Additional pre-processing:** By-products and food waste fractions are by definition not regarded as a commercial product and therefore often not handled or treated as such. Here again the very product specific nature is important to take into account, but in general the processing of such by-products requires additional steps to obtain good and clean starting materials. Waste fractions that are obtained directly from the field, from harvesting or primal selection are often dirty, covered with debris or contaminated with all sorts of unwanted matrices that first must be removed before any food-grade processing is possible. This pre-processing of the by-products or waste fractions can be a simple cleaning step, but thorough cleaning and washing requires a washing line that is often not available at the producer (farmer) and must be invested in by the processor who wishes to utilise these by-products. Since by-product processing is still often a small-scale activity with small companies that have very limited investment possibilities, collaborations with corporations or collectives are often needed. The additional investments and involvement of middlemen might annihilate the economic advantages of working with these by-products in the first place.
- **Investments:** In line with the previous bullet point, additional investments are almost impossible to exclude from the production line when working with by-products and food waste fractions. Everything depends of course on the quality of the initial by-product, if there is a need for pre-processing (cleaning, washing, removing leaves, stems or green parts…) and at what scale it needs to be done.
- **Conservation and storage:** Just like fresh products, by-products from the vegetables and fruits production are perishable and must be conserved under adapted conditions that can guarantee the best quality. Respecting the cold-chain is also very important, as stated earlier. Contamination and handling conditions must be taken into account

as they can reduce the storage duration due to a potentially higher microbiological load.

- **Partnerships with distribution chains:** Small initiatives and small-scale companies that wish to extend their product range with by-product-based foods could seek out collaborations with larger retailers and distributors to assist in the marketability of their products. Such collaborations already exist and develop further as there is a clear win-win for all involved parties. The small companies can rely on the distributors to create a market and make the products publicly known throughout their advertisement strategy and client interactions, while the distributors can offer a more versatile product range including innovations and novel sustainable foods that have been receiving much praise by a growing crowd of consumers who wish to have more control on the healthiness and freshness of food that they buy and greatly appreciate the efforts of the food industry to work towards sustainability and the elimination waste streams during the production of food.

4.2. Future perspectives

Working with by-products and food waste fractions increases the potential to maximise the commercial value of the produce and diminish the waste fractions that are generated during the various steps during production, selection and commercialisation. It opens the door for new partnerships and the development of food treatment applications that specifically deal with the by-products and their specific needs for processing and handling.

Currently the valorisation of by-products remains mostly limited to small initiatives that are focused on the short chain approach where farmers are directly selling their by-products to interested parties who utilise them for small scale productions. Some examples of small-scale initiatives in Belgium and the Netherlands are Kromkommer[1] and Wonky[2] (now a part of Syros N.V.[3]). Interestingly, the use of by-products as a selling point can be dependent on the chosen marketing strategies, with strong emphasis on the sustainable and local aspects of using by-products when targeting a specific audience, and less advertised (but not less innovative or by-product based) to appeal to a broader audience and present the by-products based foods to the mainstream markets.

The products that are obtained this way are not yet available in large retailers or supermarkets but rather at local stores and via a closed network of small retailers that seek to appeal to a target audience. It is no surprise that most of the by-product-based foods are sought after by people who consciously seek out healthy alternatives for mainstream marketed products that

[1] https://www.kromkommer.com/

[2] https://www.wonkyfood.be/

[3] https://syrosnv.com/#eat-together

may be riddled with chemicals for conservation or use unsustainable ingredients such as soybean or palm oil. In this way, the increasing amount of local initiatives can stimulate a small-scale segment in more traditional and local products and generate win-win scenarios for both the growers and the retailers.

Indeed, the growers who are producing the crops and generate a part of the by-products must directly gain from making their by-products available and if needed treat them in a manner that allows for them to be used for valorisation. A by-product must not be treated as a waste, but as a potential source of additional income. By being part of the win-win strategy growers can also invest more in the early steps of by-product valorisation, and maintain the food-grade quality of their by-products by sorting them, cooling them or even storing them under optimal conditions before selling them at a fair price to be processed by the product developers.

Working with by-products also involves additional processing and may require additional steps in the handling, cleaning and processing of the selected by-products. Investments may be needed, but what we already see is that small businesses and companies tend to collaborate with others to share the workload and create new partnerships on a local scale. Collaborations between the producing companies and sheltered workshops that can provide support in terms of hand labour or give access to storing rooms and multi-purpose washing lines are already taking off in Belgium. The next step will be an upscaling of the production (if the amounts of by-products are sufficient) and an even stronger networking with local farmers and other processors to facilitate the development and deployment of initiatives.

However, one of the major limitations to work with by-products remains the waste-fraction aspect. Prices for by-products are relatively low compared to the top class marketed product, yet this is a result of the fragile balance between offer and demand. A shift in the demand due to the development of by-product processors and retailers might create new markets which would inevitably rise the prices for the by-products, entering direct competition with the established top tier markets. Although some by-products can be obtained all year round, some will remain a seasonal product and production (with their respective prices) will be available only during certain periods, dampening the incentive of investments in machinery and infrastructure that are needed to properly process the by-products and food waste fractions.

Although the processing of by-products from the agriculture and horticulture has a promising future, and greatly contributes to the development of more sustainable and eco-friendly practices, it will most likely remain a specialised and small scale side-business that aims to increase market value and diminish waste streams and all the environmental and economic problems that go along with them. The use of by-products that are still highly qualitative, yet remain unused or underused in food applications today, has great potential to develop further into a lucrative business that not only applies to specific local niche-markets, but could also find industrial uses for the mainstream market given enough research and development can be conducted to identify the interesting by-products and develop specific processes for them.

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Appendices

Appendix 1: Poster that was made for the tomato juices developed in partnership with Tomabel and presented at the open doors day event at ILVO. The public could see how the juices were made and taste them as part of a taste panel test.