Results of the bio-based value chain apple
Laboratory analysis
1) Method

The basic research is an important skill to provide a technical overview of already investigated fields regarding the reutilisation of apple waste material and by-products. In addition, it is a basic structure for discovering of potential and novel innovations. That is the reason, why basic research was done by a mind map method with the programme Xmind 8. Meaningful mind maps are visible in the following literature research section.

By pre-defined target material and focus areas as cleaning agents and cosmetics, food additives, high tech methods and renewable products, 13 ideas were selected. Several ideas, which are not yet on the market, were chosen to investigate them on a laboratory scale. Investigated ideas are underscored in the subsequent list. The main value and necessity of laboratory activities are to verify available functionalities. All required methods and references are shown in subsequent table 1. All discovered ideas are the following:

- Wood like pellets (source of energy)
- Leather/paper
- Disposable cutlery
- Removal of dyes by biosorption
- Cat litter
- Straws
- Smoothies
- 3D food printed snacks
- Yogurt fermentation
- Dietary fibre enrichment
- Wax wrap
- Ski wax/floor treating wax
- Phenol extraction as an food additive

However, potentials of the discovered ideas will be discussed in the next chapter.

Table 1: method list of all potential ideas and feasible laboratory analysis

<table>
<thead>
<tr>
<th>scope</th>
<th>method</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different potential ideas</td>
<td>Basic research by mind mapping</td>
<td>See table 3</td>
</tr>
<tr>
<td>Raw material characterization</td>
<td>Microbiological determination of the total colony</td>
<td>International Organization for</td>
</tr>
<tr>
<td></td>
<td>count with Plate Count Agar (at 30°C for 72h</td>
<td>Standardization 2014</td>
</tr>
<tr>
<td></td>
<td>according to ISO 11133:2014) for recovery of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mesophilic bacteria to ensure quality reliability</td>
<td></td>
</tr>
</tbody>
</table>
Particle sizing conducted on a Mastersizer 2000 to determine a parameter and benchmark using apple pomace powder in different applications.  
Holland et al. 2018

Dry matter determination at 103°C until constant weight, to ensure quality requirements coupled with microbiological determination.  
Severini et al. 2018

Acid digestion in accordance a standard method to require a suitable wax structure.  
Matissek et al. 2014

Extraction of wax from apple pomace using different pre-treated raw materials to find the best application method  
Matissek et al. 2014

Smelting point to check out the wax properties and characteristics for different potential applications.  
Kofler and Kofler 1949

Wax wrap making and comparison of wax wraps made from beeswax and apple wax using the same amount and technique. Addition of plant oil can be useful to secure a better viscosity.  
https://www.beeswaxwraps.co.uk/our-wraps little-bee-fresh.de

Simply Polyphenol extraction with 60% (v/v) Ethanol at 50°C and additionally centrifugation at 4500 rpm at 4°C for 20 minutes. For a gentle extract concentration, vacuum evaporation was used.  
Benvenutti et al. 2019

Total phenol content determination with Folin Ciocalteu Reagent and Gallic acid was used as a standard solution. The measurement was done in triplicate.  
Singleton and Rossi 1965; Josuttis et al. 2010

In addition to the total colony count with a Plate Count Agar a germ differentiation was done by germ isolation and MALDI analysis.  
Wenning et al. 2014

Yogurt fermentation with different apple pomaces as a fibre enrichment using regional skim milk.  
Wang et al. 2019

A smoothie paste, containing 36.5% carrots, 45% pears, 7% kiwi, 10% broccoli and 1.5% avocado was prepared. 35% of the pear was replaced with wet apple pomace. The pastes were mixed with gelatine or AgarAgar. For safety reasons the wet apple pomace was autoclaved at 121°C for 20 minutes.  
Severini et al. 2018; Lille et al. 2018
The smoothie mass was printed in a 20mm cube and analyzed to measure the printability of the paste. The printing was performed at 30°C with 63% print speed and 70 % flow rate. It was measured the pH, dry matter content, rheological properties and texture analysis. All measurement were done in duplicate.

2) Results and potentials

This chapter describes fundamental results regarding raw material characterization, feasibility analysis for apple wax extraction, phenol extraction as food additive, yogurt fermentation with apple pomace for a dietary fibre enrichment, smoothies and 3D-printed snacks. In addition, potentials of all collected innovative ideas and the results of our basic research are described.

The total colony count, particle size and dry matter are determined as a basic raw material characterization for further product specific experiments.

a) Microbiological analysis

All apple pomace samples have been tested in duplicate on Plate Count Agar for a total bacterial count. The highest bacteria concentration with 6,27*10^4 CFU/g was found in humid apple pomace. A second humid apple pomace sample resulted in value with 1,53*10^3 CFU/g and the lowest concentration was determined in dry apple pomace with 8,77*10^2 CFU/g.

The difference in bacterial concentration between humid and dry apple pomace is logically comprehensible. The bacterial growing was minimized due to the drying process. The diversity of bacterial contamination in various humid apple pomaces is explainable with different processing steps, storage conditions and raw material properties.

b) Dry mass determination

The dry mass value is an additional parameter to ensure and maintain the microbial stability of apple pomace and resulting products. All measurements were done in double identifications. From these data, the average value was calculated. Humid apple pomace own a dry value of 61,67 %, dried apple pomace 93,26 % and dried, grinded apple pomace 91,55 %.
c) Particle sizing

Particle sizing conducted on a Mastersizer 2000 was determined for three different grinding steps of dried apple pomace. The D[0,5]-values range from 487,981 to 346,105 µm. Lower D-values indicate smaller particle sizes. It is depending on the approach and experimental requirements, which particle sizes are needed.

d) Apple wax extraction

i) Extraction with suitable pre- and subsequent treatments

For a wax extraction from apple pomace the common Soxhlet extraction method was used. In addition, as pre-treatment, an acid digestion was done according to Matissek et al. 2014 for improving the wax structure and increasing the yield as shown in the following result table.

Table 2: comparison of wax yields from different apple pomaces and treatments

<table>
<thead>
<tr>
<th>pomace</th>
<th>treatments</th>
<th>yield %</th>
<th>characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humid</td>
<td>Extraction by Soxhlet</td>
<td>1,82</td>
<td>yellowish, wax-like structure, porous, good smell</td>
</tr>
<tr>
<td>Dried</td>
<td>Extraction by Soxhlet</td>
<td>3,79</td>
<td>Yellowish/greenish, wax-like structure, less porous, more sticky, good smell</td>
</tr>
<tr>
<td>Dried and grounded</td>
<td>Extraction by Soxhlet + acid digestion</td>
<td>3,47</td>
<td>Orange/beige, been wax-like structure, no intensive smell, less sticky</td>
</tr>
<tr>
<td>Dried and grounded</td>
<td>Extraction by Soxhlet + acid digestion + rotary evaporator</td>
<td>3,67</td>
<td>Orange/beige, been wax-like structure, no intensive smell, less sticky</td>
</tr>
</tbody>
</table>

In the study of Günther Lang et al. 1996, they have obtained comparable results in testing different apple pomace extraction methods for an usage in the cosmetic sector. According to our performed experiments, a higher yield is reachable with industrial dried and depectinized apple pomace. Equally, subsequent treatment of the wax extract with a vacuum evaporator had a positive effect on the yield. The used particle size with a D(0,5)-value of approximately 346 µm has been measured with a Mastersizer 200. For a better wax structure, the used acid digestion with 25% hydrochloric acid is responsible.
ii) Melting point

The melting point is an important parameter to determine wax properties and characteristics for different potential applications as wax wraps, ski wax or for floor treating agents. All samples were measured in a double determination by a Kofler hot bar. The substance azobenzene and been wax are used as reference material. The determined melting point of beeswax was 70°C, apple wax (without pre-treatment) 71°C and apple wax (with pre-treatment) was 53.5°C.

The result interpretation of the determined melting points depends on different potential applications.

iii) Making of wax wraps

Wax Wraps are promising and an ideal alternative to plastic wraps to preserve food. Usually, they are made from bee’s wax, which is quite expensive, and therefore a limited factor. The idea behind this experiment was to replace bee’s wax with extracted wax from apple pomace. For a direct comparison, wax wrap models from both raw materials (bees and apple wax) were made with the same amount and method.

The result shows differences in colour and haptic properties. The layer of the wrap from apple wax was softer and more agile. From this, it can be concluded that apple wax can be an additional raw material for making wax wraps and an alternative of plastic.

e) Phenol extract as food additive

A polyphenol extract extracted from apple pomace can be used as food additive in various products. For example the corporation of polyphenol grape pomace extracts in yoghurt was already tried because of a possible improvement of starter culture microorganism during the fermentation process (Azevedo et al. 2018).

The result of first polyphenol extraction experiments shows a low yield of a low yield of 1.38g Gallic acid equivalent / kg apple pomace. The efficiency and realization of profit has to be assessed individually depending on the effort and feasibility of each eligible company.
f) Yogurt fermentation

An addition of apple pomace in the yogurt production has a potential for a dietary fibre enrichment and can influence the texture in a positive way. May the in pomace contained polyphenolic compounds can enhance the beneficial health effects of milk-derived functional foods (De Souza de Azevedo et al., 2018). The result of the germ differentiation by MALDI-TOF shows us the necessity of suitable hygiene measures and/or after treatments directly by the manufacturer.

g) Smoothie and 3D food printed snacks

The printed smoothie snacks are a possibility to increase the consumption of healthy food, due to a healthy composition of the snacks. Regarding to first impressions, the printing quality can been improved with the added apple pomace, especially the viscosity and firmness. The viscosity influence can be detected with rheological measurements and the firmness with a Texture analyser.

A meaningful outcome of all performed experiments in the laboratory is the finding that a high efficiency can only be reached with the usage of the entire by product. Preceding processes like extraction can decrease the efficiency through further costs and time exposure.

3) Literature research

In the following table 3 there are shown all results of possible innovative ideas and collected potentials.

Table 3: results of additional discovered potential ideas with apple pomace

<table>
<thead>
<tr>
<th>scope</th>
<th>potential</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ski wax</td>
<td>- alternative of ski waxes, containing organofluorine compounds</td>
<td>Nordic Ecolabbeling 2018; Rogowski et al. 2005</td>
</tr>
<tr>
<td></td>
<td>- possible changing regulations regarding ski wax compositions</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Flow diagram for a visualisation of the polyphenol extract yield efficiency, outcoming of first laboratory experiments
<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floor treating wax</td>
<td>- Possible maintenance and polishing ability</td>
<td>Smith and Murphy 1986</td>
</tr>
<tr>
<td></td>
<td>- Natural and degradable wax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Wax amount of various floor treating and cleaning agents could be replaced with apple wax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Good odor of apple wax</td>
<td></td>
</tr>
<tr>
<td>Wood like pellets</td>
<td>- Alternative energy source</td>
<td>Verma et al. 2012</td>
</tr>
<tr>
<td></td>
<td>- Agro pellets for domestic heating boilers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Apple pellets yielded the highest combustion efficiency (91.3%) after wood pellets (92.4%)</td>
<td></td>
</tr>
<tr>
<td>Leather/paper</td>
<td>- Natural imitation leather that does not represent any risk for the environment</td>
<td>Alberto Volcan 2009</td>
</tr>
<tr>
<td></td>
<td>- Bio-degradable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Advantages: not inflammable and not toxic</td>
<td></td>
</tr>
<tr>
<td>Disposable cutlery</td>
<td>- Plastic replacement: sustainable alternative with minimal impact when the source material is obtained in a sustainable manner</td>
<td>Gautam and Caetano 2017; Razza et al. 2009</td>
</tr>
<tr>
<td></td>
<td>- Potential product for a usage in everyday life</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Already existing cutlery from for example areca palm and coconut tree</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Bio-degradable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Interesting for example for fast food restaurants, canteens and festivals</td>
<td></td>
</tr>
<tr>
<td>Removal of dyes by biosorption</td>
<td>- Removal potential of different dyes used in various industry sectors (textile, food, plastics, paper)</td>
<td>Robinson et al. 2002</td>
</tr>
<tr>
<td></td>
<td>- Alternative of the normally used expensive active carbon</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- For the removal of dyes from a synthetic effluent 1g of apple pomace with a particle size 600 µm is suitable</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Description</td>
<td>Sources</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Cat litter        | - Alternative for bentonite cat litter  
                    - Already existing alternatives are animal litter made from wood, clay and peanut hulls  
                    - Wood cat litter has following positive properties: absorbs moisture very quickly, binds odours, ecologically and compostable  
                    Stephen L. Ivie 1992;  
                    Raymond J. Fry 1974                                                                                                                        |
| Straws            | - Plastic replacement  
                    - Changing in regulation (implementation of prohibition)  
                    - Bio-degradable  
                    - Interesting for restaurants and various catering sectors                                                                                   | https://wisefood.de/                                                                                                                                |
| dietary fibre     | - Non-caloric bulking agents for partial replacement of flour (carbohydrates), fat and sugar  
                    - Apple dietary fibre granules  
                    - Increased content of phenolic compounds after fermentation of dried apple pomace in bread production                                          | Galanakis 2018; Struck et al. 2018; Quiles et al. 2018; Rodríguez Madrera et al. 2015                                                          |
| enrichment        |                                                                                                                                                                                                           |                                                                                               |
a) Basic research

The following mind maps are important results of our basic research:

Figure 2: First mind map of apple with all important involved fields which are important for a potential analysis

All involved fields shown in the mind map (figure 2), are the basis for the development of novel innovations and further scientific analysis. It appears that apple, regarding this matter, is a mature saturated market. The focus of the detailed research goes especially to the three-outlined points: market, production and processing with a cross-industry character for being more open-minded regarding other industry sectors.

Figure 3: Apple mind map with production and processing in focus
Further research is focused on production and processing to achieve technical impact and to detect how potential products can be defined. There are still a lot of possibilities to reuse apple waste materials and by-products.

Figure 4: Apple mind map with potential products in focus

The outcome of this research is that the ideal target material for an innovation process is apple pomace because it is available in large quantity as a waste product in the juice industry. The technical screening depends on which kind of reuses and upcycling possibilities already exists with apple pomace and especially where still potentials are.

The whole list of potential ideas shown in chapter 1 are outcomings from the basic research.
4) Publication bibliography


Benvenutti, Lais; Bortolini, Débora Gonçalves; Nogueira, Alessandro; Zielinski, Acácio Antonio Ferreira; Alberti, Aline (2019): Effect of addition of phenolic compounds recovered from apple pomace on cider quality. In LWT 100, pp. 348–354. DOI: 10.1016/j.lwt.2018.10.087.

De Souza de Azevedo; Aliakbarian; Casazza; LeBlanc; Perego; de Souza Oliveira (2018): Production of fermented skim milk supplemented with different grape pomace extracts: Effect on viability and acidification performance of probiotic cultures. In PharmaNutrition (6).


Matissek, Reinhard; Steiner, Gabriele; Fischer, Markus (2014): Lebensmittelanalytik. Berlin, Heidelberg: Springer Berlin Heidelberg.

Quiles, Amparo; Llorca, Empar; Schmidt, Carolin; Reißner, Anne-Marie; Struck, Susanne; Rohm, Harald; Hernando, Isabel (2018): Use of berry pomace to replace flour, fat or sugar in cakes. In Int J Food Sci Technol 53 (6), pp. 1579–1587. DOI: 10.1111/ijfs.13765.


Rodríguez Madrera, Roberto; Pando Bedriñana, Rosa; Suárez Valles, Belén (2015): Production and characterization of aroma compounds from apple pomace by solid-state fermentation with selected yeasts. In LWT - Food Science and Technology 64 (2), pp. 1342–1353. DOI: 10.1016/j.lwt.2015.07.056.


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