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Design of Animal Hygiene Products with Regard to Odour and Moisture Absorption

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Affidavit

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Abstract

Inorganic cat litter materials made from bentonite have a tremendous impact on the environment. In recent years consumers got more and more interested in alternatives to conventional clay litter. Sawdust is a remainder of the wood industry and can be used as bulk material in organic cat litter types. In the present work the prototype of an organic cat litter material based on spruce sawdust was successfully developed. Additives increasing clumping ability, malodour absorption and antimicrobial effect were identified. Development was started at laboratory scale using a household mincer to simulate the pelleting process. Scale-up was carried out in a pilot scale and an industrial scale pelleting plant. Different types of starch as well as guar gum, carob gum, xanthan gum, carboxymethylcellulose and other additives were investigated for their clumping properties. Among all tested additives xanthan gum was observed to be the most suitable additive for this purpose. The water uptake capacity of the developed cat litter material was in the range of 30 g to 40 g of dry litter per 50 mL of deionized water. Comminution of the produced cat litter pellets was carried out using a cutting mill, a crusher and a ball mill. None of the tested methods showed ideal particle size distributions. Either too many large or too many fine particles were produced. However, the determined results provide a good starting point for further fine tuning of the particle comminution procedure. Based on information about odour active substances present in cat urine available in scientific literature, synthetic cat urine authentically mimicking its odour was prepared. It was composed of 3-mercapto-3-methylbutan-1-ol, 4-methyl-4-mercaptopentan-2-one, 4-methylphenol, 2,3-benzopyrrole, 2-(methylthio)ethanol, 2-methyl-3-furanthiol, ammonium chloride, ammonium hydrogen carbonate, disodium oxalate and urea at different concentrations. Increasing the xanthan gum concentration in the cat litter prototype increased the odour absorption ability of the material. Compared to existing organic and inorganic cat litter brands the uptake of maldours could be significantly improved. Microbial growth on the cat litter surface was investigated using *E. coli*, *Bacillus subtilis*, *Micrococcus ssp.*, *Trichoderma ssp.* and *Aspergillus brasiliensis*. None of the bacterial strains was viable on the prototype cat litter samples. The same result was observed for *Trichoderma ssp.*, a mold fungus. A general growth inhibition of microorganism due to the low water activity of the litter material was observed. The growth of *Aspergillus brasiliensis* could be limited by increasing the xanthan gum concentration and therefore further decreasing the water activity of the litter. Alternatively, cationic starch Cationamyl 9865 was observed to have an antifungal effect. In the present work novel insights in the sensory properties of wood based cat litter and its odour absorption ability were presented. Sensory evaluation of organic cat litter by a sensory panel and quantification of microbial growth on the surface of organic cat litter were carried out for the first time.

Zusammenfassung

Anorganische Katzenstreusorten basieren meist auf Bentonit und können daher keiner Abfallverwertung zugeführt werden. Diese Materialien müssen auf Deponien gelagert werden und stellen so eine außergewöhnlich hohe Belastung für die Umwelt dar. Um dem entgegen zu wirken wünschen sich viele Konsumenten Katzenstreusorten, die auf natürlichen Materialien wie Holz basieren. Sägespäne bieten dafür die geeignete Grundsubstanz und sind in holzreichen Ländern wie Österreich in ausreichender Menge vorhanden. Die vorliegende Arbeit behandelt die erfolgreiche Entwicklung eines Katzenstreuprototypen, dessen Hauptbestandteil Fichtenholzsägespäne sind. Zusätzlich wurden Additive zur verbesserten Klumpenbildung, Geruchsbindung und antimikrobiellen Wirkung untersucht. Erste Versuche zur Katzenstreupelletierung wurden in einem Fleischwolf durchgeführt. Später wurde die entwickelte Katzenstreu in Pilotpelletieranlagen und im Industriemaßstab produziert. Verschiedene Stärkearten, Guarkernmehl, Johannisbrotkernmehl, Xanthan, Carboxymethylcellulose und viele weitere Additive wurden auf ihre Verklumpungseigenschaften getestet. Xanthan wurde dabei als der am besten geeignete Zusatzstoff identifiziert. Die Ergiebigkeit der entwickelten Katzenstreusorten lag zwischen 30 g und 40 g trockener Streu, die zur Aufnahme von 50 mL deionisiertem Wasser benötigt wurden. Mithilfe einer Schneidmühle, durch Quetschung und in einer Planetkugelmühle wurden die hergestellten Pellets zerkleinert. Keine der verwendeten Methoden lieferte eine für die Anwendung als Katzenstreu ideale Korngrößenverteilung. Es wurden entweder zu viele große Partikel oder zu viele feine Partikel generiert. Allerdings liefern die gezeigten Ergebnisse einen guten Ausgangspunkt für weitere Untersuchungen in diese Richtung. Die geruchsaktiven Verbindungen im Katzenurin sind in der einschlägigen Fachliteratur beschrieben. Auf Basis der dort verfügbaren Informationen wurde ein synthetischer Katzenurin hergestellt, der das Geruchsprofil echten Katzenurins authentisch nachbildet. Dem synthetischen Katzenurin wurden 3-Mercapto-3-methylbutan-1-ol, 4-Methyl-4-mercaptopentan-2-on, 4-Methylphenol, 2,3-Benzopyrrol, 2-(Methylthio)ethanol, 2-Methyl-3-furanthiol, Ammoniumchlorid, Ammoniumhydrogencarbonat, Natriumoxalat und Harnstoff in verschiedenen Konzentrationen zugesetzt. Durch Steigerung der Xanthankonzentration in der Katzenstreu konnte die Geruchsbindung verbessert werden. Verglichen mit am Markt erhältlichen Katzenstreumarken war die Intensität des wahnehmbaren Katzenuringeruchs im entwickelten Katzenstreuprototypen signifikant niedriger. Mikrobielles Wachstum auf der Oberfläche der Pellets wurde mithilfe der Teststämme *E. coli*, *Bacillus subtilis*, *Micrococcus ssp.*, *Trichoderma ssp.* und *Aspergillus brasiliensis* untersucht. Keine der Bakterienspezies konnte auf den Katzenstreupellets überleben. Dasselbe gilt für *Trichoderma ssp.*, einen Schimmelpilz. Hervorgerufen durch die niedrige Wasseraktivität des Streumaterials wurde eine generelle Hemmung des mikrobiellen Wachstums beobachtet. Erhöhen der

Xanthankonzentration in der Katzenstreu, sowie durch die Verwendung der antifungal wirkenden, kationischen Stärke Cationamyl 9865 konnte das Wachstum von *Aspergillus brasiliensis* weiter eingedämmt werden. Neue Einblicke in die sensorischen Eigenschaften von holzbasierten, organischen Katzenstreusorten und deren Geruchsbindungseigenschaften konnten durch die in der vorliegenden Arbeit gezeigten Ergebnisse gewährt werden. Zum ersten Mal wurde organische Katzenstreu von einem Expertenpanel sensorisch evaluiert, sowie eine Quantifizierung des mikrobiellen Wachstums auf der Oberfläche derartiger Produkte durchgeführt.

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1 Introduction

In times of global warming and environmental pollution being a ubiquitous problem, many industrial sectors are looking for alternative solutions to make their products more environmentally friendly. At the same time a growing number of people gets aware of their user behaviour and optimizes their personal ecological footprint. This idea has also made an appreciable progress in comparatively small sectors like pet keeping. Especially pets living in flats require the use of litter material that takes up the pets' urine and faeces and also absorbs the arising malodours. However, commercially available litters are often made of inorganic material and can only be disposed via the residual waste. Inorganic cat litter cannot be incinerated and has to be stored at landfill sites. According to a report published by the city of Vienna, 30 000 t of inorganic cat litter are used per year for the 200 000 cats living in Austria's capital [14]. Substitution of the inorganic cat litter by an organic product would on the one hand decrease the amount of landfill in Austria and on the other hand increase the amount of waste that can be used for energy production by biomass combustion. Even though organic cat litter types are sometimes advertised as being compostable or providing the possibility to be disposed via the toilet, authorities often forbid to do so. The city of Graz neither allows to discard cat litter via the organic waste nor via the waste water system [16, 17]. However, Austria's organic waste directive basically allows the usage of sawdust, chopped wood as well as liquid and solid animal faeces for the production of high quality compost [12].

Inorganic cat litter is often made from bentonite, which is a limited resource produced mainly in Canada, Northern America and Spain. The use of organic litter material can make cat owners more independent from the international market and the high demand for bentonite, which is also used in construction industry [14].

As Austria and especially Styria is rich in wood and wood remainders like sawdust, there is a great opportunity to manufacture a type of cat litter that is produced regionally and sustainably but also convinces the consumer with its properties. The specific weight of wood is much lower compared to that of bentonite [14]. Therefore it is more comfortable to transport, especially if people are going by foot, by bike or when using public transport. In addition to that the structure of wooden fibers is well suited for the uptake of watery liquids [7, 14, 38].

The aim of the present work is to make the production of cat litter based on wood sawdust possible and to ensure consumers' requirements on cat litter. Above all, the clumping ability of the litter material must be guaranteed, but also the water uptake capacity and the odour absorption are important criteria during decision for a particular product. Therefore the odour active substances in cat urine are

identified by an extensive literature research. Afterwards sensory evaluation to investigate the odour absorption ability of the produced samples is performed. The pellet production process itself is first tested at laboratory scale in a mincer and further investigated at a pilot scale pelleting plant. Microbial growth behaviour at the pellet surface is studied and additives having antimicrobial effects are identified. Additionally, the sensory properties of the litter material are evaluated by a sensory panel. Finally, the most promising recipes are produced at industrial scale.

2 Theoretical Background

As only in Austria's capital Vienna approximately 200 000 individuals live, cats are ranked among the most popular pets [14]. Many of these cats are kept indoors or combined indoors and outdoors, which results in the majority of cats being offered to use a cat litter box. Here the question arises, which properties the litter material should have. Besides clumping ability, many cat owners name odour absorption as selection criterion for a specific cat litter brand [14]. With inorganic cat litter, microbial growth on the pellet surface has never been an issue. However, with bio-based litter material this needs to be considered. In the scientific literature as well as in a lot of patents it is claimed that at least one of these requirements is fulfilled.

In this section the current state of knowledge considering the chemical composition of cat urine, microorganisms associated with cats and cat litter as well as possible additives in organic cat litter is summarized. Additionally, the principles of performing sensory evaluation using a sensory panel are explained.

2.1 Chemical Composition of Feline Urine

The diet of domestic cats consists mainly of meat, as they are considered to be true carnivores. Therefore the pH of their urine is slightly acidic. However, the composition and the pH value of feline urine differs if the diet is changed. Conventional canned food is usually heat sterilized. Even though this process does not change the overall amount of amino acids, it indeed increases their bioavailability. Some amino acids such as methionine are known to lower the pH value of feline urine. [8]

Cottam et al. reported differences in the urine composition of male and female cats. They investigated parameters like the pH value, protein, uric acid, ammonia, calcium, magnesium and phosphate concentrations. A total number of 45 male cats and 26 female cats were examined. The pH of female cats' urine is 5.97 on average and has only a small range between 5.54 and 6.57. At the same time male cats show a far larger range of 5.73 to 7.39, which results in a mean pH value of 6.37. The average protein content is 375 mg/L for males and 305 mg/L for females. Urea has the highest concentration value of a single compound with 1386 mmol/L for males and 1295 mmol/L for females. Urine of male cats contains 0.52 mmol/L of uric acid on average, whereas 0.39 mmol/L are found in female cats' urine. Ammonia shows the second highest concentration of a single compound: 118 mmol/L is the determined mean value in male cats' urine and 121 mmol/L are found in urine of female cats. Calcium, magnesium and phosphate are present to smaller extents with values of 0.69 mmol/L, 3.11 mmol/L and 81 mmol/L in males and 0.82 mmol/L, 3.94 mmol/L and 77 mmol/L in females. [8]

2.1.1 Odour Active Substances in Feline Urine

Different papers published in the 1950s, 60s and 70s report that the distinctive tom-cat odour is androgen dependent and can be significantly reduced by castration of male cats [3, 26, 32]. However, the source of the tom-cat odour was not clear at first [3]. At the same time, a new substance referred to as the "cat spot" was discovered via paper chromatography in the urine of domestic cats [11, 58]. It was identified as a new sulfur-containing amino acid called felinine [58]. Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) is assumed to be a precursor molecule to a pheromone [21, 22, 36, 42]. It is involved in territorial marking, however, its specific biological function is still not completely clear [22, 23, 36, 42]. Degradation products of the cat specific felinine were found to be the source of the very specific cat urine smell [22, 27, 31].

Felinine excretion is sex dependent [22, 41]. In the urine of male cats higher felinine levels (2.0 g/L on average) are found than in the urine of females (0.3 g/L on average) [21,22,36,42]. In the urine of kittens no felinine is detected, independent of their sex [22, 36, 41]. A female kitten does not show any felinine in its urine until attaining sexual maturity. After that the same felinine excretion rate as in adult female cats is found for this kitten. In addition to that, Roberts could increase felinine levels in female cats by testosterone treatment. However, treating male cats with oestrogen did not affect their felinine excretion levels. [41] According to Hendriks et al., male cats excrete an average of 122 mmol of felinine per kg of bodyweight per day via the urine. Castrated males, entire and spayed female cats excrete an average of 41 mmol, 36 mmol and 20 mmol per kg of bodyweight per day. Statistical evaluation resulted in significantly different felinine excretion amounts between entire and castrated males as well as castrated males and spayed females. No statistically significant difference could be detected between entire and spayed females. [23]

The sulfur containing amino acids methionine and cysteine are both precursor molecules of felinine, whereas the latter is quantitatively more important [21]. The enzyme cauxin is a carboxylesterase regulating the secretion of felinine. It was shown in vitro that cauxin cleaves the precursor molecule 3-methylbutanol-cysteinylglycine to felinine and glycine. The production of cauxin is also age and sex dependent, providing a further explanation why entire male domestic cats produce higher amounts of felinine compared to females or castrated males. [36]

It was observed that the development of the typical cat urine odour is time dependent [22, 27, 45]. Fresh cat urine is almost odourless, but after 12 h to 24 h the most pungent cat urine smell is perceived [22, 27]. In addition to that, the odour level was dependent on the diet and the period within the cat's sexual cycle [22, 27]. This might be due to the fact that felinine excretion also shows a biphasic excretion pattern following the two breeding seasons during the year [49]. One group of scientists investigated degradation of felinine via chemical reactions [42], whereas others focused on the enzymatic decomposition of the amino acid [27, 45].

Rutherfurd et al. found that synthetic felinine reacts with urea, but not with

other nucleophiles present in cat urine such as ammonia. The reaction of urea and felinine takes place over a wide pH range of 3 to 10. At least 100 mmol/L urea are necessary to degrade all felinine, and at a urea concentration of 30 mmol/L 50 % of the present felinine vanished. Products of this reaction were analyzed using mass spectrometry, which indicated that carbamyl-felinine, cystine, cysteine and carbamyl-cysteine are possible reaction products together with two unidentified peaks at 79.97 m/z and 99.78 m/z. [42]

In the work published by Joulain and Laurent it is assumed that odour active degradation products of felinine like 3-mercapto-3-methyl-1-butanol (MMB) arise due to microbial action or oxidation by air contact [27]. MMB at a concentration range of 100 ppb to 1000 ppb is observed to be a major contributor to the typical cat urine smell [22,27]. Mattina et al. identified MMB in bobcat urine too, together with other sulfides, disulfides and trisulfides derived from felinine [31]. Miyazaki et al. and Starkenmann et al. published detailed analysis of the head space gas of domestic cat urine [36, 45]. Besides MMB, 3-mercapto-3-methyl-butyl formate, 3-methyl-3-(methylthio)-1-butanol and 3-methyl-3-(2-methyldisulfanyl)-1-butanol were identified. These cat specific odorants are assumed to be degradation products of felinine. However, it remains unclear how these compounds are achieved from felinine. [36] Traces of 4-methyl-4-mercaptopentan-2-one (MMP), which is one of the most important odour active substances in cat urine [27,45], were found in aged tom-cat urine [27]. Mixtures of MMB and MMP in the right ratio give a realistic cat urine smell, indicating that these two substances are key odorant volatiles [45].

A recent work by Starkenmann et al. elucidates the effect of a mixture of common soil bacteria incubated with the urine of entire male and female cats as well as castrated males and spayed females. It was observed that felinine is degraded by enzymes belonging to the group of β-lyases. The C-S bond in the α-position of the free amino acid is cleaved and MMB is released. In total, nineteen different odour active substances were identified in the head space gas of fermented cat urine, but not all of them are important impact odours for the cat urine smell. Especially MMB, MMP, 3-methyl-3-(methyldithio)-1-butanol, 3-methyl-3-(methylthio)-1-butanol, 2-(methylthio)ethanol (MTE), (+/-)-3,7-dimethyloct-3-sulfanyl-6-en-1-ol, 2-methyl--3-furanthiol (MFT), 4-(methylthio)-2-butanone and 3-mercapto-3-methyl-butyl-formate contribute to the sulfury smell. These compounds are described using one or more of the following attributes: meaty, sulfury, skunky, fried onion, sweat, cabbage, asparagus, tropical fruit, citrus, blackcurrant. As the odour threshold of most sulfury compounds is very low, quantification is difficult. However, in the urine of neutered cats 5×10^{-3} mg/L 3-methyl-3-(methylthio)-1-butanol were found, and intact males excreted 1.5 mg/L. Besides the above mentioned substances also sulfur free molecules like indole or p-cresol contribute to the typical malodour of aged cat urine. Cat urine contains approximately 0.05 mg/mL to 0.1 mg/mL of p-cresol and indole. [45]

Interestingly, blackcurrant aroma is sometimes associated with the specific “catty” odour [27, 46]. This is most likely due to the fact that tertiary thiols such as MMB, MMP and 3-mercapto-3-methyl-butyl formate occur in cat urine [27, 45]

and some foods too [2]. MMB and MMP can be found in wine (especially Sauvignon blanc and Scheurebe), roasted coffee, blackcurrant, hop, citrus and grape fruit juice [2,4,10,19,45,50,51]. At low concentrations, many tertiary thiols have a fruity scent, whereas at high concentrations they are perceived as cat urine smell [2]. For example, highly diluted MMP is described as having a strong blackcurrant flavour, rather than a urine smell [27, 45]. Besides concentration dependent changes, also mixing effects of several odour active substances can have severe impact on the aroma quality [2].

Literature focusing on odour active substances in cat urine is very limited. However, due to the fact that some substances are also found in foods and beverages, odour threshold values (OTV) of most cat urine specific compounds are available. The odour recognition threshold value (ORTV) is defined as the lowest concentration at which a substance can be identified in a certain medium [2,4,53]. The odour detection/perception threshold value (ODTV or OPTV) is the minimum concentration of a substance in a certain medium, below which one is not able to detect the difference from the control sample [10]. For the OPTV it is not necessary to identify the compound [2, 53]. It is typically lower than the ORTV [2]. Odour threshold values can vary significantly depending on the medium used [4].

Van Gemert found that MMB has an odour detection threshold value of 2 µg/kg to 6 µg/kg of water [53], whereas Blank and Tominaga et al. reported that the perception threshold of this compound in water is 1.3 µg/kg [4, 50] and 1.5 µg/kg in an aqueous 12 vol% ethanol/water solution [4, 50, 51]. The smell of MMB is described as broth-like [4] or similar to cooked leeks [35, 50, 51]. The aroma of MMP is described as sulfury [4], blackcurrant- [4], box tree- [35, 51], guava- [35], cat urine- [35], broom- [35, 51] and passion fruit-like [35]. The odour perception threshold is much lower than that of MMB. In water an OPTV of 10^{-4} µg/kg was determined by various authors [2, 10, 35]. In a 10 wt% ethanol/water solution the OPTV reported by Guth is 6×10^{-4} µg/L [19], in a 12 vol% ethanol/water solution the OPTV is 8×10^{-4} µg/L [51] and 10^{-3} µg/kg in a 10 vol% ethanol/water solution [4]. The reported odour perception threshold value for MMP in wine ranges from 8×10^{-4} µg/L to 3×10^{-3} µg/L [10, 35].

MTE has an aroma decribed as French beans [35], meaty, sulfury, skunky and fried onions [45]. Its ODTV is 120 µg/kg in water [53].

MFT has a meaty [2, 4], roasty and sulfury smell [4]. Its ORTV is 7×10^{-3} µg/L in water [2, 4]. Van Gemert reported an ORTV of 5×10^{-3} µg/kg to 0.01 µg/kg in water for this compound [53].

3-Mercapto-3-methyl-butyl formate has a sulfury, catty odour [4]. Blank and Belitz et al. reported an ORTV of 3×10^{-3} µg/kg in water [2, 4]. The ODTV in water ranges from 2×10^{-3} µg/kg to 5×10^{-3} µg/kg [53], a value very close to the odour recognition threshold value.

The ODTV of indole ranges from 11 µg/kg to 140 µg/kg in water [53]. Cresol is formed via microbial degradation of tyrosin. It has an ORTV of 55 µg/kg in water. Its odour is described as smoky. [2]

2.2 Cat-Associated Microorganisms

Bacteria carried by companion animals or pets are investigated by Buma and coworkers. They collected samples from healthy cats and especially from their front paws, the fur at the lumbar region and the anal region. In addition to that, the cat toilets were examined. A group of seven cats, consisting of four females and three males, was investigated. All cats were allowed to move freely indoors and outdoors. The authors also ensured that none of the cats suffered from a skin disease which could falsify the result. [5]

As a measure for the number of bacteria present, the number of colony forming units (CFU) was determined. The cats' fur contained a total number of 9.6×10^5 CFU/g of hair, each front paw hosts 3.8×10^4 CFU and in the anal region 3.1×10^5 CFU are found per unit of sampling area. Some strains living on the cats' fur could be identified, e.g. *Micrococcus ssp.*, *Staphylococcus cohnii* and other *Staphylococcus ssp.*, *Bacillus cereus* and other *Bacillus ssp.*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pseudomonas ssp.*, *Sphingobacterium multivorum*, *Xanthomonas maltophilia*, yeast and other gram-positive and gram-negative rods. The front paws hosted the following bacteria: *Micrococcus ssp.*, *Staphylococcus caprae* and other *Staphylococcus ssp.*, *Bacillus ssp.*, *Pseudomonas ssp.*, *Xanthomonas maltophilia*, *Sphingomonas paucimobilis*, yeast and other gram-positive and negative rods. From the anal region of the cats many *Staphylococcus* species like *S. auricularis*, *S. caprae*, *S. cohnii*, *S. epidermidis* and *S. hyicus* were isolated. In addition to that, *Bacillus ssp.*, *Escherichia coli*, *Pseudomonas ssp.*, *Flavobacterium oryzihabitans* and *F. meningosepticun*, *Shingomonas paucimobilis* as well as other gram-positive and negative rods were found. In the cat toilets *E. coli* and other coliforms as well as aerobic bacteria are found to high extents. *Staphylococcus aureus* was not detected. [5]

Similar to other mammals, the urine of healthy cats is sterile and bacteria isolated from cat litter originate mainly from the animals' fur, paws and faeces [5]. However, many companion animals and pets suffer from urinary diseases. If more than 10^5 microorganisms are found per mL of cat urine, a urinary tract infection is present. In case of such a diagnosis *Escherichia coli*, *Proteus ssp.*, *Staphylococcus aureus*, *Streptococcus ssp.* and *Pasteurella multocida* were isolated most frequently from infected cat urine. [59] In a different study mainly *E. coli* was detected in urine of cats suffering from urinary diseases. In addition to that, *Enterococcus faecalis* and the coagulase-negative strain *Staphylococcus faelis* were found in high concentrations. *Proteus ssp.*, *Enterobacter ssp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* are detected to minor extents. [30]

2.3 Innovations in Organic Cat Litter and its Additives

The demand on biologically degradable cat litter is steadily rising due to different reasons. Less environmental impact is the most important one, but also the lower

specific weight plays a role for many people. Several inventions concerning organic cat litter were already patented, some of them focusing on the clumping behaviour, whereas others try to improve the odour absorption or to reduce the microbial activity.

Dried distillers grain, which is a remainder of the ethanol industry, can take up large amounts of water. Together with guar gum as clumping agent and glycerol as a dust retardant it is used in a recipe for organic cat litter. Glycerol also helps to bind the guar gum particles to the dried distillers grain. Head space concentrations of odour active sulfur molecules are reduced by copper sulfate pentahydrate. 3-Mercapto-2-butanol, which is a molecule that is structurally very similar to the odour active MMB present in cat urine, is used as test compound. Compared to the control sample, 67.3 % of the head space concentration of 3-mercapto-2-butanol could be reduced by the reaction with copper sulfate pentahydrate. As copper sulfate is used in fish tanks and ponds to prevent the growth of algae and other microorganisms, it can be assumed that copper sulfate would also show some antimicrobial action in cat litter. However, this hypothesis was not checked by the authors of this publication. [54]

The method of using copper salts for the binding of sulfur containing malodorants is also well known in wine fabrication. Thiols contribute severely to the typical cat urine smell, but are present in some types of white wine too. MMP for instance can be found in cat urine and also in Sauvignon blanc. All thiols can react with copper ions to form insoluble copper thiolates. [43] This could be a promising possibility in terms of odour absorption in organic cat litter. On the other hand, copper sulfate pentahydrate should not be ingested by animals or small children who could get access to cat litter accidentally. [54]

A patent filed by Buttersack and Wullbrandt describes the invention of organic cat litter based on dried sugar beet pulp. Clumping additives like cellulose derivatives, such as carboxymethylcellulose (CMC) and galactomannans, such as guar gum are used. The authors advise that not more than 10 % CMC and 5 % guar gum should be added. Starches can be used alternatively to guar gum or CMC. The authors preferred starches or starch derivatives that are soluble in cold water. Besides the additives, the particle size distribution (PSD) is important for the clumping behaviour. The authors tried to obtain the same PSD for all batches of their cat litter. Their strategy to control the absorption of malodours is more unspecific. To reduce odours, up to 20 % of inorganic substances like kieselguhr, zeolithes or bentonite are added. These are all substances with a huge specific surface area that should cage odour active substances deep inside the cat litter pellets. [6]

Besides the presence of different thiols, the formation of ammonia is the most important source of bad smell of cat toilets. Often strong acids, such as sulfuric acid are used to bind ammonia to insoluble ammonium sulfate. Sometimes the litter material contains pressure sensitive fragrance capsules that break and release perfume when the animal steps onto the litter. In the approach described by Heitfeld and Wood, the enzyme urease is inhibited by the addition of guanidine salts (e.g. guanidine hydrochloride), alkali metal fluroides (e.g. sodium fluoride), alkali

metal bisulfites (e.g. sodium bisulfite) and mixtures thereof. Urease, orginating from microorganisms, decomposes urea. During this reaction ammonia is formed. Guanidine hydrochloride acts as a competitive inhibitor of urease as the structure of the salt is very similar to the structure of urea. Sodium fluoride and sodium-bisulfite inhibit urease too. Additionally, sodium bisulfite maintains a low pH value, helping to inactivate urease. This approach resulted in a significant reduction of ammonia release. [20]

The same idea but a different strategy is used by Reddy and Sloan. They inhibit the growth of urease positive bacteria by applying urease negative bacteria to the cat litter. Among others, different species of the genera *Lactococcus*, *Streptococcus*, *Pediococcus*, *Propionibacterium*, *Leuconostoc* and *Lactobacillus* are used for this purpose. As a result, the enzyme urease is not active and urea is not degraded, which results in a significant reduction of odour active ammonia. [39]

Besides agricultural side products, wood and wood remainders such as sawdust are widely used base materials for organic cat litter. Rettenmaier suggests that at least 30 wt% of the total mass of the litter should be wood particles. An average granule size of 4.5 mm is required for a high water uptake rate. However, at least 25 % of the particles must not exceed a maximum dimension of 3 mm. For the particle size evaluation the Feret diameter is determined via dynamic image analysis. Knowledge about the particle size distribution of the litter material is not only important for the liquid uptake rate but also for the ease of user handling, since small and light particles tend to stick to the cat's fur or paws. This effect is prohibited by manufacturing particles with a larger average size. Clumping of the fibrous sawdust particles is ensured by addition of at least 3 wt% of cellulose or starch derivatives and plant gums like guar, tara or carob gum. [40]

A mixture of wood and ground grain as basic litter material is used by Hughes and coworkers. The material contains between 1 wt% and 50 wt% wood particles and 50 wt% to 99 wt% ground grain. 95 % of the litter particles need to have a diameter between 125 µm and 4 mm. The preferred mixture contains ground wheat grains and aspen particles. As a dust retardant vegetable oils such as soybean oil are used. No further clumping additives are described. [24]

Another commercially available organic cat litter type is based on milled, extruded corn grains. It is claimed that the manufacturing process is responsible for the high specific surface area of the granular material and its high water uptake rate. No additional clumping agents are necessary for the formation of stable clumps. Due to the large water binding capacity, the odour absorption ability is high. The material weighs only one third in comparison to bentonite. This reduces costs for transport and waste treatment, which also makes the product more environmentally friendly. Dust is reduced by sieving. The bulk density of the material needs to have a value between 350 g/L and 450 g/L. It is preferred to use whole grain corn only. However, mixtures of whole grain corn and whole grain wheat with the majority being made up by whole grain corn are also possible to use. As no further additives are used, this material has the advantage of being completely safe in case of accidental ingestion or inhalation by animals or small children. [15] On

the contrary, using food or feedstuff as cat litter material should be scrutinized too.

Weaver filed a patent about clumping organic cat litter with yellow pine as its basic material. One or more clumping agents and a non-ionic surfactant are added. The surfactant with an amount between 1 wt% and 5 wt% increases the water uptake rate. CMC in combination with guar gum is used as clumping additive. Weaver suggests to use CMC with a viscosity of 8000 mPa s as the clump formation is faster and the resulting nuggets are more stable. Due to the fact that CMC is very expensive only 1 wt% to 2 wt% are used. If guar gum is the only clumping agent at least 10 wt% to 15 wt% must be added. The preferred mixture contains more than 90 wt% wood material, between 1 wt% and 2 wt% CMC and between 3 wt% and 6 wt% guar gum. The author claims that the described product is fully biodegradable, has improved moisture uptake and natural odour control properties. [57]

Guar gum is used in various inventions considering biodegradable cat litter. However, this additive has suffered from severe price increase as it is also used for hydraulic fractioning and the overall production volume decreased lately. Therefore cheaper alternatives like xanthan gum, plantago gum, methyl cellulose, pectin, lignin, camelina and lesquerella seedmeals, waxy corn starch, high amylose- and normal corn starch-sodium palmitate inclusion complexes are tested by Vaughn and coworkers. Xanthan gum, methyl cellulose and plantago gum were identified as equally good clumping additives for organic cat litter. Xanthan gum shows the highest clumping percentage of all compounds studied. It performs even better than guar gum. Even though also the price of xanthan gum increased recently, it is considered to be a good alternative to guar gum. [55]

Antimicrobial action of cat litter was realized by Lezdey and Lezdey. The litter material described contains an admixture consisting of a diquat, which is a biocidically active diquarternary ammonium compound, a polycarboxilic acid, at least one phenol compound and a carrier which is either water or a water/alcohol mixture. A polycarboxilic acid is a carboxylic acid compound carrying two to four carboxylic acid groups or anhydrides, acting as polycarboxylic acid or salts thereof. The inventors claim that the described mixture acts against a variety of odour causing microorganisms. For odour absorption, basic compounds like ammonium or alkali metal carbonate, bicarbonate, phosphate or perborate can be used. [29]

2.4 Sensory Evaluation Using a Sensory Panel

Sensory evaluation is defined as the method of measuring, analyzing and interpreting responses to products perceived via seeing, smelling, tasting, hearing and touching [52]. The use of sensory panels to perform detailed flavour evaluation is a generally accepted technique and widely used for foods, beverages and many other consumer products [44, 52].

Basically two different types of sensory panels exist, the consumer panel and the expert panel [44, 48]. The consumer panel consists of a group of untrained people, representative for the target audience of a tested product. Consumer panels

are mostly used for market research, whereas expert panels are used for analytical questions. [44] The members of an expert panel get trained depending on the requirements of the analytical problem [33, 44]. For the statistical evaluation a minimum number of expert panel members is required depending on the test procedure, but due to the specific training six to thirty panelists are usually enough. Consumer panels need to be much larger than that. [44]

Tasks handled by the consumer panel are called hedonic questions. These test procedures focus on the personal preferences of the panelists. Sensorically skilled persons like members of expert panels are not allowed to work on hedonic questions, as their training level makes unbiased tasting impossible. [44] On the contrary, consumer panels cannot deal with discriminative tests, descriptive and quantitative-descriptive procedures [44, 48]. In discriminative studies the members of the expert panel have to decide whether the present samples show any differences or not [44]. During a descriptive test the properties of a sample have to be described by using a previously obtained vocabulary [33, 44]. Quantitative-descriptive methods combine qualitative description of a sample via specific wording with quantitative assessment of the intensity of its sensory properties [44, 48]. However, all of these test procedures are based on statistical methods in a way that the results of the single panel members can be evaluated and a statistically verified result for the whole panel can be derived [44]. Sensory evaluation should take place in a sensory laboratory which provides identical conditions for all panel members [33, 44]. To prevent biased tasting, the samples should be coded with a three-digit random code and the presentation order should be randomized [1, 33, 48].

As the use of a sensory panel requires the participation of humans, some ethical standards have to be considered. This includes that the organizer of the test has to guarantee for the safety of all panel members by avoiding any chemical or microbial hazard. [52] In return, some basic principles should be met by the sensory panel members. It is of utmost importance that the panelists are motivated and show commitment to this activity. In addition to that, panelists must be able to focus on particular sensory tasks and avoid certain eating habits prior to a test session. [37, 44] Shortly before a sensory evaluation smoking needs to be avoided too. Different physical and psychological factors such as diseases, stress or partial taste- or odourblindnesses can have a negative impact on a panel member's work. If this is the case, the panel leader should ask the affected panel member to temporarily leave the sensory panel. [44]

As a descriptive analysis and a ranking test with subsequent Friedman analysis were performed in this study, these two procedures are explained in more detail.

Descriptive Analysis

The aim of a descriptive analysis is to fully characterize a product according to its perceived sensory properties [37]. This method is useful for the development of a new product, for the improvement of a product or process, to train a panel for further testings or to do quality control [1, 37].

Descriptive analysis is among the most powerful tools in sensory evaluation as it can be combined with e.g. consumer preference investigations. For instance this provides the possibility to derive knowledge about the desired properties or the desired composition of a certain product. Descriptive methods always require an expert panel that is to some extent trained on this type of evaluation. [37] However, the degree of training strongly depends on the complexity of the investigated samples. In a study on Pinot noir wine McDaniel et al. investigated the aroma differences occurring when fermenting the grapes with different strains of malolactic bacteria. The expected differences between the samples were very small and therefore extensive training including the development of 33 specific aroma descriptors was necessary. For less challenging questions, the use of general descriptive terms might be sufficient. [33] Basically, it needs to be ensured that all panel members possess the same vocabulary and use the same wording for specific sensory properties [44].

Ranking Test

The purpose of a ranking test is to evaluate the difference of several samples related to a single property [1]. A small number of samples (usually four to six [44]) are coded and presented simultaneously to each panel member [1]. The panelists are asked to rank the samples according to the intensity of the investigated property [1].

Ranking tests can be performed by the consumer panel as well as the expert panel [1,44]. If a consumer panel is used, the samples are usually ordered according to the personal preference of each judge [44]. Expert panels rank the samples according to the intensity of a specific sensory property, e.g. the sweetness [1,13,44]. In case of k samples, the rank positions 1 to k are assigned. If two of the samples could not be distinguished, the same half rank can be assigned twice, e.g. two times 3.5 instead of 3 and 4. [13] One possibility to statistically evaluate the rank orders of all panel members is the Friedman test, which allows to determine whether all samples are identical with respect to the investigated property or if a significant difference between the samples is observed [13,44]. In addition to that, the difference between two selected samples can be determined [13]. The exact algorithm is shown in section 3.9.

3 Materials and Methods

3.1 Chemicals and Equipment

Additives Used for Pelleting Tests

For laboratory scale pelleting tests pea starch and mallow shred of unknown purity were used. They were donated by Hasslacher Norica Timber. Food grade potato starch, corn starch and carrageen were purchased from Spar Austria. Cationic starch and carboxymethylcellulose were both technical grade. They were kindly provided by Stefan Spirk from the Institute of Paper, Pulp and Fibre Technology of Graz University of Technology. Carboxymethylcellulose was purchased from Sigma Aldrich. Wheat starch was available from previous pelleting experiments. Food grade sodium alginate and calcium lactate were kindly provided by Professor Erich Leitner, head of the Institute of Analytical Chemistry and Food Chemistry. The two reagents have the commercial names Algizoon and Calazoon and were purchased from Biozoon. Apple pectin, guar gum, carob gum and xanthan gum were all food grade and of the brand Natura. All laboratory scale experiments were performed using a Kenwood mincer of type 885 (320 W). The perforated disc of the mincer had a thickness of 5 mm and a hole diameter of 6 mm.

During the pilot scale pelleting tests at Wieselburg two different cationic starch types called Cationamyl 9854 and Cationamyl 9865 were used. Both were donated by Agrana Austria. The referring product data sheet can be found in the appendix. Food grade potato starch was purchased from Spar Austria. Xanthan gum, guar gum and carob gum were obtained from BuXtrade. They were all food grade. Activated carbon (purity 100 %) was purchased from Roth. Fumed silicon oxide (purity 99.7 %) was purchased from VWR Chemicals. The silica particles had a size of 100 mesh.

In the industrial scale pelleting process cationic starch of type Sobocat, kindly provided by Südstärke, was used. The product data sheet can be found in the appendix. Pelleting starch of unknown composition and purity was available at Hasslacher Norica Timber Preding. Food grade xanthan gum was obtained from Neupert Specialities. The particle size of this xanthan gum type was 80 mesh. The product data sheet can be found in the appendix. The dosing system was borrowed from Bioenergy 2020^+ Wieselburg. Its settings can be found in the appendix in table 5.1.

All chemicals were used without further purification.

Odour Imitating Synthetic Cat Urine

3-Mercapto-3-methylbutan-1-ol (purity 98 %) and p-cresol (purity 100 %) were obtained from Sigma Aldrich. Indole (purity 100 %) was purchased from TCI Chemicals. 4-Methyl-4-mercaptopentan-2-one (purity 98.8 %) was obtained from Endeavor Chemicals. 2-(Methylthio)ethanol (purity 99 %) and 2-methyl-3-furanthiol (purity 95 %) were purchased from Sigma Aldrich. Ammonium chloride (purity 100 %) was purchased from Merck. Ammonium hydrogen carbonate (purity 100 %) was purchased from Sigma Aldrich. Disodium oxalate (purity 99 %) and urea (purity 99.5 %) were obtained from Roth. All chemicals were used without further purification.

Water Uptake Capacity of Cat Litter

Five commercially available products were used. *Commercial Organic Cat Litter 1* and *Commercial Inorganic Cat Litter 1* were purchased from OBI Austria. *Commercial Inorganic Cat Litter 2* and *Commercial Organic Cat Litter 2* were purchased from dm Austria. *Commercial Organic Cat Litter 3* was kindly provided by Hasslacher Norica Timber. All other samples were produced during the pilot scale pelleting experiments. The plastic container used was purchased from OBI Austria and had a volume of 5 L. Its dimensions were 290 mm × 290 mm × 120 mm.

Pellet Comminution and Particle Size Analysis

Crushing was performed by Hasslacher Norica Timber. Cutting was performed using a Retsch SM100 cutting mill at the Institute of Analytical Chemistry and Food Chemistry. A sieve insert of the cutting mill with a hole size of 8 mm was used for all experiments. Milling was done using a Retsch PM100 planetary ball mill at the Institute of Process and Particle Technology at Graz University of Technology. The volume of the milling cup was 200 mL. Eight milling balls were used. The milling cup as well as the milling balls were made from agate stone. Sieving was performed using a Fritsch Analysette type 03.200 sieve tower. The mesh sizes of the used sieves were 4 mm, 2 mm, 1.6 mm and 0 mm. Different sieves were used in the sieving analysis performed by Hasslacher Norica Timber. In this case the sieve sizes were 3.15 mm, 2.8 mm, 2 mm, 1.4 mm, 1 mm, 0.8 mm, 0.5 mm, 0.25 mm and 0 mm.

Microbiological Experiments

Pure cultures of *E. coli*, *Bacillus subtilis*, *Micrococcus ssp.*, *Aspergillus brasiliensis* and *Trichoderma ssp.* were kindely provided by the University of Applied Sciences Joanneum Graz. Peptone water and blood agar plates were purchased from VWR. The product data sheet can be found in the appendix. For determination of the antimicrobial effect of cationic starch in liquid peptone water cationic starch type Cationamyl 9865 provided by Agrana Austria was used. For the other experiments

cat litter pellets produced during the pilot scale pelleting tests were used. Ethanol (70 %) was used for cleaning and sterilization of the triangular cell spreader. All chemicals were used without further purification. Determination of the optical density of the overnight cultures was performed in a Varian spectrophotometer at a wavelength of 600 nm.

Sensory Evaluation

Freshly prepared odour imitating synthetic cat urine, as described in section 3.4, was used. Cat litter types produced during the pilot scale pelleting tests and commercially available products, mentioned earlier, were investigated.

3.2 Laboratory Scale Pelleting Tests

A mincer was used to simulate the processes happening during pellet production. Spruce sawdust was used as the basic material in combination with the various additives listed in table 3.1.

Table 3.1: Tested additives and additive blends, their concentration in wt% of the sawdust c, the mass of the additives $m_{additive}$ and the sawdust $m_{sawdust}$, the volume of deion. H_2O V_{water} and the heating time $t_{heating}$.

Additive	c [wt%]	$m_{additive}$ [g]	$m_{sawdust}$ [g]	V_{water} [mL]	$t_{heating}$ [min]
Pea Starch	2	0.89	44.9	200	15
	5	0.54	27.0	100	15
	15	0.44	22.0	100	15
Potato Starch	1	0.21	21.3	75	15
	2	0.42	21.3	60	15
	5	1.08	21.5	60	15
	10	2.17	21.7	60	15
Cationic Starch	1	0.21	20.8	50	15
	2	0.41	20.6	50	15
	5	1.04	20.8	60	15
	10	2.05	20.5	50	15
Corn Starch	1	0.21	21.0	100	15
	2	0.44	22.0	50	15
	10	2.10	21.0	50	15
Wheat Starch	1	0.23	22.7	75	15
	2	0.43	21.6	75	15
	5	1.01	20.2	65	15
	10	2.14	21.4	65	15
Apple Pectin	5	1.00	20.7	50	15

CMC[1]	1.5	0.60	40.4	100	0
Guar Gum	10	2.00	20.9	70	0
Carob Gum	10	2.00	20.9	70	0
Xanthan Gum	0.5	0.25	50.9	150	0
	1	0.20	20.0	50	0
	2	0.40	20.4	50	0
	5	2.00	39.4	100	0
	10	2.00	20.9	60	0
Na^+ Alginate Ca^{2+} Lactate	0.5	0.20 1.47	40.0	100	0
	1	0.20 0.70	20.9	50	0
	2	0.40 0.70	20.6	50	0
	5	1.00 0.70	20.9	50	0
	10	2.00 0.70	20.7	50	0
Carrageen Carob Gum	2.5 2.5	0.50 0.50	20.2	50	15
Guar Gum Carob Gum	5 1 3 1.5 2.5 2.5	2.00 0.40 1.20 0.60 1.00 1.00	40.3 40.7 40.0 	100 80 80 	0 0 0
Xanthan Gum Carob Gum	4.5 0.5 4 1 3 2 2.5 2.5	1.80 0.20 1.60 0.40 1.20 0.80 1.00 1.00	40.3 40.8 40.6 40.9 	80 90 90 90 	0 0 0 0
Mallow Shred Cat. Starch[2]	25 5 25 10	5.00 1.25 5.00 2.60	20.2 20.6 	70 70 	20 20

	4.5	1.80	40.6	100	10
	0.5	0.20			
	4	1.60	40.3	100	10
Xanthan Gum	1	0.40			
Cat. Starch	3	1.20	40.8	100	10
	2	0.80			
	2.5	1.00	40.7	100	10
	2.5	1.00			
Xanthan Gum	2	0.80	40.8	100	0
CMC	1	0.40			

[1] Carboxymethylcellulose [2] Cationic Starch

Per batch 20 g or 40 g of pure spruce sawdust were weighed and filled into a beaker glass. Especially with additives requiring thermal activation 20 g of sawdust should not be exceeded. Otherwise difficulties providing a homogeneous heat distribution within the sample arise.

The additive or the additive blend was weighed and mixed with dry sawdust. The necessary amount of deionized water was quantified using a graduated cylinder and added to the mixture. Depending on the type of additive the amount of water had to be varied to homogeneously wet the sample and at the same time avoid unabsorbed water. Per 20 g of sawdust at least 50 mL of deionized water were used to fulfill this requirement. If a larger amount of water was necessary, it was added in steps of 5 mL or 10 mL. The sample was stirred until a homogeneously wetted mixture was achieved. Some additives require thermal activation. In these cases the mixture was heated on the hotplate until the temperature distribution throughout the sample was homogenous and a minimum temperature of 60 °C was obtained. This was ensured by measuring the temperature of the material at different areas of the beaker glass using a conventional laboratory thermometer.

Afterwards the paste was filled into the mincer. The perforated disc of the mincer was used for compaction and sizing of the pellets. The produced pellets were collected in aluminum trays. Drying was carried out over night in a drying chamber at 105 °C. The pellets were evaluated with regard to size, shape and stability.

3.3 Pilot Scale Pelleting at Bioenergy 2020+ Wieselburg

Properties of an industrial pelleting process could be reliably simulated at the pilot scale pelleting plant at Bioenergy 2020+ Wieselburg. Above all, the additives' behaviour and the impact of the additive concentration were of interest.

3.3.1 Initial Pilot Scale Pelleting Test

During the first pilot scale experiments different additive types were tested. On the one hand, single compounds were added at different concentrations. On the other hand, blends of two additives were used. In addition to that, the interaction of substances with different swelling properties was investigated. Additives reacting only with hot water like potato starch or the cationic starch type Cationamyl 9854 were mixed with substances that show swelling when being contacted with cold water. Such additives are xanthan gum, carob gum, guar gum and cationic starch type Cationamyl 9865. Pellets consisting only of spruce sawdust without any additive were produced as a reference.

Eight batches with a mass of 20 kg per batch were produced. Before loading the sample material into the pelleting machine it was homogeneously mixed by a concrete mixing machine. Like in the lab scale experiments sawdust and additives were blended in dry state. Depending on the moisture content of the sawdust a defined amout of water was added using a pressure sprayer. Water was added under constant stirring to avoid aggregation of the material.

The moisture of the sawdust was checked regularly to guarantee a water content in the range of 13 % to 14 % throughout all batches. After incubating the sample material for approximately 1 h at room temperature it was filled into the pellet press. To prevent contamination between different batches the first 5 kg of each batch were discarded. The pellets were collected in plastic containers and cooled for several hours. Per batch 15 kg of pellets were packaged.

3.3.2 Subsequent Pilot Scale Pelleting Test

The aim of the subsequent pilot scale pelleting test was a closer investigation of the additives that showed promising properties throughout the first experiment. Above all, different concentrations of xanthan gum, blended with cationic starch type Cationamyl 9865, were tested. Two samples containing either activated carbon or fumed silicon oxide were produced too. Due to their large specific surface area activated carbon and silicon oxide serve as cage substances and are therefore assumed to improve the odour absorption. Again a reference batch only containing spruce sawdust was produced.

Eight batches were pressed. The samples were prepared as described in subsection 3.3.1.

3.4 Industrial Scale Pelleting Test

Fabrication of cat litter pellets in an industrial pelleting machine was investigated at Hasslacher Norica Timber Preding. For the experiments pure spruce sawdust was used as the base material. Each batch contained 0.5 wt% of pelleting starch. In addition to that, cationic starch of type Sobocat and xanthan gum were used as additives. Seven batches including a reference batch were produced.

The throughput of the pelleting machine was 2300 kg/h. Prior to the experiment the moisture content of the sawdust was determined to be 12.28 %. The additives were added in dry state via the dosing system. The pelleting process took 15 min per batch. The pellets were filled into Big Bags and cooled outside for 10 min. A sample of approximately 10 kg was withdrawn from every Big Bag. These samples were evaluated in the laboratory. In addition to that, the moisture of the pellets was measured in % as well as the bulk density in kg/m^3. These data can be found in the appendix in table 5.1.

3.5 Fabrication of Odour Imitating Synthetic Cat Urine

In the currently available literature the number of quantitative results for the odour active substances of feline urine is very small. Many authors argue that the concentration levels of these substances are low, making exact quantification difficult. However, the key impact odorants could be identified and are reported in several publications (cf. subsection 2.1.1).

Prior to the preparation of the synthetic cat urine, stock solutions of p-cresol, indole, MMB, MMP, MTE and MFT were made. Methanol was used as solvent. The concentration of each stock solution was 1 g/L.

As water is the bulk component in urine, deionized water was used as basic matrix for the preparation of an odour imitating synthetic cat urine. Deionized water was filled into a 1 L volumetric glass flask. Urea, ammonium chloride, ammonium hydrogen carbonate and disodium oxalate were added based on the extents described by Cottam et al. and in the product data sheet of a commercially available odourless cat urine imitation sold by Synthetic Urine. This product contains <0.4 % ammonium chloride, <0.5 % ammonium hydrogen carbonate and <0.01 % disodium oxalate. The solid compounds urea, ammonium chloride, ammonium hydrogen carbonate and disodium oxalate were completely solved in the provided volume. Per 1 L of water 84 g of urea, 4 g of ammonium chloride, 5 g of ammonium hydrogen carbonate and 0.1 g of disodium oxalate were added. This refers to the above mentioned data of the commercially available product and the mean urea concentration of 1386 mmol/L in the urine of male cats published by Cottam and coworkers [8].

The odour threshold values described in subsection 2.1.1 were used as a starting point to create an odour mimicking the odour of aged cat urine. In a stepwise manner aliquots of MMB, MMP, MTE, MFT, p-cresol and indole stock solutions were added until an odour signature similar to cat urine is reached. Aliquotes of the stock solutions were transferred to the volumetric flask using disposable capillary pipettes made from glass. 70 µL MMB, 50 µL MMP, 400 µL MTE, 10 µL MFT, 60 µL p-cresol and 60 µL indole stock solution were necessary per 1 L of deionized water. After all components were added the volumetric flask was filled up with deionized water. The final concentrations of the above mentioned compounds in the prepared synthetic cat urine were: 1.4 mol/L urea, 74.8 mmol/L ammonium

chloride, 63.2 mmol/L ammonium hydrogen carbonate, 0.7 mmol/L disodium oxalate, 0.58 µmol/L MMB, 0.38 µmol/L MMP, 0.55 µmol/L p-cresol, 0.51 µmol/L indole, 0.09 µmol/L MFT and 4.34 µmol/L MTE.

A sample of this solution was prepared on a paper strip and presented to cat keeping members of the expert panel for evaluation. Even though urine originating from cats contains a much broader spectrum of chemical compounds, a realistic cat urine-like odour profile was received. Therefore the solution described here can be considered as an odour imitating synthetic cat urine. The solution had to be kept sealed and cooled in the fridge. It had to be consumed within a few days as the odour changes its properties over time.

3.6 Water Uptake Capacity of Cat Litter

According to the regulations given in ÖNORM S 1002, the specific water uptake capacity of clumping cat litter needs to be measured [47]. The ÖNORM S 1002 was declared to be invalid in 2015, but no follow-up document was available. Therefore the slightly adapted procedure described below was applied.

5 L of sample material were filled into a plastic box of the dimensions 290 mm × 290 mm × 120 mm. Commercially available clumping cat litter or own sample material was used. In case of self-produced cat litter samples the pellets were comminuted using a Retsch SM100 cutting mill prior to the water uptake capacity analysis. A titrating burette was fixed to a stand and filled with 50 mL of deionized water. The ÖNORM S 1002 requires that the burette is totally discharged within 15 s. However, due to the small opening of the used burette this took approximately 25 s. Therefore a measuring cylinder was used to produce litter clumps in addition to the burette. This measuring cylinder was emptied within 15 s, measured with a stopwatch. Duplicates were produced for each method.

The clumps were incubated for 30 min at room temperature to achieve proper hardening. A cat litter sieve scoop was used to withdraw the clumps from the plastic box and to separate unused litter particles. Afterwards, the clumps were weighed. For the evaluation of the experiment the weight of the used water was subtracted from the total weight of the clump. To accurately calculate the mass of the water volume used per clump the density of water at 25 °C, 997.04 kg/m^3, was considered [18]. Thus, the amount of litter necessary for absorbing 50 mL of deionized water was determined.

3.7 Pellet Comminution and Particle Size Analysis

Crushing, cutting and milling were investigated for pellet comminution. *Commercial Organic Cat Litter 1*, *Commercial Organic Cat Litter 3* and the crushed 2 wt% xanthan gum cat litter were visually evaluated prior to the particle size analysis.

The particle size distribution (PSD) of *Commercial Organic Cat Litter 1*, 5 wt% xanthan gum litter and 2 wt% xanthan gum litter was analyzed at Hasslacher Nor-

ica Timber Preding. The 5 wt% xanthan gum pellets were comminuted using the Retsch SM100 cutting mill. The 2 wt% xanthan gum litter was crushed by Hasslacher prior to the sieve analysis. *Commercial Organic Cat Litter 1* was sieved without any further treatment to compare the particle size distribution obtained from different comminution methods with the PSD of a commercially available product. A sieve tower with different sieves was used. The empty sieves were weighed. Samples were placed at the top sieve. Sieving is continued until no mass variation was visible on top of each sieve. The sieves were weighed again and the particle mass on top of each sieve was calculated.

The 7 wt% xanthan gum litter was cut by the Retsch SM100 cutting mill prior to the experiment. This sample was sieved at the Institute of Process and Particle Technology at Graz University of Technology. Sieving was performed as described above. An additional sample of the same litter type was comminuted by a Retsch PM100 planetary ball mill to investigate different grinding methods. 57.8 g of the 7 wt% xanthan gum litter were filled into the milling cup together with eight milling balls. The sample was milled for 1 min. Evaluation was only carried out optically. No further sieve analysis was necessary.

3.8 Microbiological Experiments

3.8.1 Growth Behaviour at the Pellet Surface

The ability of microorganisms to grow on the surface of wooden cat litter containing different additives was investigated. A selection of the microorganisms mentioned in section 2.2 was used for this purpose.

Suspensions of bacteria and fungi were prepared in peptone water, a rich medium. An aliquot of each suspension was applied onto the surface of the litter samples and incubated for several days at room temperature. Afterwards, the litter samples were resuspended in fresh rich media and mixed thoroughly. An aliquot of the supernatant was plated onto an agar plate and incubated again at room temperature. The grown colonies were counted and the germ number was determined. As different volumes of resuspension medium and different pellet masses were used the number of colony forming units (CFU) was calculated per mL of resuspension volume and per g of pellet mass. The referring formulas can be found below.

$$\frac{CFU}{\mathrm{mL}} = \frac{n_{\mathrm{colony}}\, DF}{V_{\mathrm{plated}}} \tag{3.1}$$

In the equation above n_{colony} refers to the number of colonies visible on the petri dish, DF is the dilution factor of the plated volume and V_{plated} is the volume of the supernatant plated out on the surface of the agar.

$$\frac{CFU}{\mathrm{g}} = \frac{CFU}{\mathrm{mL}}\,\frac{V_{\mathrm{media}}}{m_{\mathrm{pellets}}} \tag{3.2}$$

Here V_{media} is the total volume of medium used for resuspension of the pellets and m_{pellets} is the mass of these pellets.

Initial Investigation of Growth Behaviour

For the preliminary test three different bacteria strains and one mold fungus were used. *E. coli*, *Micrococcus ssp.*, *Bacillus subtilis* and *Aspergillus brasiliensis* were grown separately on cat litter types containing 5 wt% xanthan gum, 2.5 wt% Cationamyl 9865 and 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9854 and 2.5 wt% xanthan gum as well as on a reference litter made from spruce sawdust without additives.

For each strain of microorganism an overnight culture (ONC) was prepared in a 10 mL glass tube with screw plug. Under sterile conditions, close to the flame of a bunsen burner, fresh peptone water was filled into the glass tube and some cell material was transferred from the pure culture agar plate into the media using an inoculation loop. The inoculation loop was flame sterilized and cooled prior to the transfer. A sterility control was carried out. This was done by dipping the sterilized and cooled inoculation loop into a tube containing fresh, sterile peptone water. All test tubes were incubated over night at 37 °C in a heating block. Constant stirring was provided by teflon-coated agitators (500 rpm). To improve the heat transfer the empty space between the heating block and the test tubes was filled with deionized water. After the incubation period the optical density of the overnight cultures was determined using a photometer at a wavelength of 600 nm (OD_{600}). This method provides the possibility to quantify the cell density.

The cat litter samples were comminuted with the Retsch SM100 cutting mill. Sterilization for at least 3 h at 110 °C dry heat was performed in a drying chamber. Sterilization using saturated steam was not possible in this case as the material could swell and the morphology of the samples would be changed. The sterilized sample material was filled into petri dishes. The empty petri dishes as well as the filled ones were weighed to determine the pellet mass.

For the inoculation of one petri dish, 1 mL of bacteria or fungi ONC was evenly distributed on the surface of the sample material. One sterility control per litter type was prepared using 1 mL of sterile peptone water, instead of ONC. In addition to that, one growth control per strain was carried out to ensure that the overnight cultures contain living microorganisms. Therefore 100 µL of bacteria or fungi ONC were plated onto agar plates using a triangular cell spreader made from glass. 100 µL of sterile media were plated onto a blood agar plate as sterility control.

The plates and petri dishes containing bacteria were incubated in the drying chamber at 40 °C. The fungi samples were kept in a polystyrene box at room temperature. After 24 h the temperature of the drying chamber rose to 45 °C. Therefore the bacteria samples were further incubated in the polystyrene box. After a total incubation time of 72 h the agar plates containing the growth controls were evaluated.

After six days of incubation (144 h) the pellet samples were resuspended in a

mixture of heat sterilized deionized water and fresh media. After thorough stirring, 100 µL of each supernatant were plated onto blood agar plates. 100 µL of a 100-fold dilution of each supernatant were plated onto blood agar plates too. All plates were incubated in the polystyrene box at room temperature. The growth status was checked once every 24 h. After one week of incubation the plates were evaluated.

Follow-Up Test with Reduced Number of Strains

The number of test strains was reduced from four to three. The gram-negative bacteria *E. coli*, the gram-positive bacteria *Bacillus subtilis* and the mold fungus *Aspergillus brasiliensis* were grown on the cat litter types containing 5 wt% xanthan gum, 2.5 wt% Cationamyl 9865 and 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9854 and 2.5 wt% xanthan gum as well as on a reference litter made from spruce sawdust without additives.

The experiment was performed as described above. Overnight cultures and one sterility control were prepared. The optical density OD_{600} was measured. Pellet filled petri dishes were inoculated with 1 mL of bacteria or fungi suspension. All samples and controls were incubated in the polystyrene box at room temperature. The growth control plates were evaluated after an incubation period of 96 h. Once every 24 h the growth status of all plates was checked. After five days of incubation (120 h) the pellets were resuspended in sterile peptone water. 100 µL aliquots of the supernatants were plated onto sterile agar plates. After six days (144 h) of incubation at room temperature the growth status was documented.

Growth Behaviour at Different Amounts of Cationic Starch

To investigate the effect of cationic starch on the growth behaviour of filamentous fungi, *Trichoderma ssp.* and *Aspergillus brasiliensis* were grown on cat litter types containing 5 wt% xanthan gum, 1 wt% Cationamyl 9865 and 5 wt% xanthan gum or 2.5 wt% Cationamyl 9865 and 2.5 wt% xanthan gum.

Overnight cultures, OD_{600} measurement, inoculation of pellet filled petri dishes as well as the preparation of sterility and growth controls was performed as described above. After six days of incubation at room temperature pellet samples were resuspended in sterile media. Supernatant aliquots were plated on blood agar plates and incubated at room temperature for seven days. After this incubation period the plates were evaluated.

Growth Behaviour Depending on Type and Amount of Additive

The aim of this experiment was to find a correlation between the total additive concentration of the litter samples, the relative amount of cationic starch and the growth behaviour. *Aspergillus brasiliensis* was grown on cat litter types containing 7 wt% xanthan gum, 5 wt% xanthan gum, 1 wt% Cationamyl 9865 and 5 wt% xanthan gum as well as 2.5 wt% Cationamyl 9865 and 2.5 wt% xanthan gum.

The experiment was carried out as described earlier. *Aspergillus brasiliensis* was grown on the pellet samples for seven days. The litter material was resuspended in sterile peptone water. Supernatant samples were plated on blood agar plates and incubated at room temperature. After 96 h of incubation the growth status was checked. The plates were sealed with parafilm to slow down fungal growth. After a total incubation time of six days the agar plates were evaluated.

3.8.2 Antimicrobial Effect of Cationic Starch in Liquid Media

To prove the antimicrobial effect of cationic starch, 1 %(w/v), 3 %(w/v) and 5 %(w/v) of Cationamyl 9865 were dissolved in sterile peptone water. 3 %(w/v) could be dissolved completely, whereas 5 %(w/v) dissolved only partially. In addition to that, a positive and a negative control were carried out. The positive control contained sterile media without cationic starch, inoculated with *Aspergillus brasiliensis*. Sterile media without cell material was used as negative control.

The test tubes were either inoculated with cell material by a flame sterilized inoculation loop or in case of the negative control the sterilized inoculation loop was dipped into the liquid without fungi material. The tubes were closed with a screw plug and put into a heating block. The fungi cultures were incubated for 48 h at 37 °C. Constant stirring at 500 rpm was provided.

After incubation, 100 µL of each test liquid were plated onto sterile blood agar plates. In addition to that, a 10-fold dilution of each culture and the positive control was prepared using fresh media. 100 µL of each dilution were plated on agar plates too. All agar plates were incubated in the polystyrene box at room temperature. After 96 h the plate surfaces were checked for fungal growth. Incubation was continued for seven additional days. After a total period of eleven days (264 h) the plates were evaluated.

3.9 Sensory Evaluation of Cat Litter

To evaluate the sensory properties of cat litter two different sensory methods, using an expert panel, were performed. The expert panel consists of persons trained on handling different analytical sensory problems (cf. section 2.4). No specific training was necessary for working on the subsequently described procedures. A descriptive characterization of different cat litter samples was carried out to make the panelists familiar with the wooden matrix of the organic cat litter and the odour of the synthetic cat urine. Therefore it was sufficient to use general descriptive terms. No special vocabulary had to be trained. The second sensory method was a ranking test that was statistically evaluated using the Friedman analysis. Test logs for both methods can be found in the appendix.

The sample preparation was the same for both procedures and follows the one described in section 3.6. Evaluation of the samples was always performed by smelling. At first cat litter pellets were comminuted using the Retsch SM100 cutting mill.

Figure 3.1: a) Test installation at seat 1 for a sensory evaluation. b) Seat arrangement in the sensory laboratory.

5 L of the cut product or a commercially available sample were filled into a plastic container (dimensions: 290 mm × 290 mm × 120 mm). Clumps of the cat litter were produced with a titrating burette. Per clump 10 mL deionized water or synthetic cat urine were used. After 30 min of incubation the clumps were sufficiently hardened and could be taken out of the plastic box by a sieve scoop. Each clump was transferred into a white 250 mL plastic cup that was closed with a transparent plastic lid. All samples were labeled with a random three-digit code to ensure blind tasting. The number of samples prepared was at least as high as the number of people registered for the test session to avoid that one sample had to be tasted twice in succession by two panel members. This could significantly change the properties of the clump and of the head space above the clump. During the test session one sample after another was opened and evaluated by smelling. Afterwards the sample was closed immediately to avoid biasing the odour of the next sample. All sensory tests were performed under standardized conditions in a sensory laboratory (cf. figure 3.1). The arrangement of the seats can be seen in image section b) of figure 3.1 and the test installation is shown in image section a) of figure 3.1. Samples were always presented in randomized order to avoid the fist-sample effect. This means that each panelist receives the samples in a different order. The three-digit code as well as the sample order was generated via Compusense, a software for sensory evaluation. Between eight and thirteen panelists participated in the sensory evaluation sessions.

For the descriptive analysis two clumps per type of cat litter were prepared. One of these clumps was produced with 10 mL deionized water, whereas the other one contained 10 mL synthetic cat urine. The aim of this test procedure was to make the panelists familiar with the cat litter samples, to determine the sensory properties of

the sawdust in combination with the additives and to get information on the odour differences perceived if synthetic cat urine was used or not. Eight panelists took part in this experiment. Three different cat litter types produced during the first pilot scale pelleting test were analyzed. These samples contained 5 wt% xanthan gum, 2.5 wt% xanthan gum and 2.5 wt% Cationamyl 9854 or 10 wt% guar gum and 5 wt% carob gum. Additionally one product from retail, *Commercial Organic Cat Litter 1*, was analyzed. Descriptive terms characterizing the samples were noted down on the test log by each panelist. For each sample all mentioned descriptive terms were collected and counted. Similar terms were grouped, e.g. wood and sawdust. However, all terms contributing to one group are mentioned in the group name. Evaluation was performed with regard to the frequency of the descriptive terms used for the individual samples.

All ranking tests were carried out using four different cat litter samples. Clumps were prepared using 10 mL of synthetic cat urine. The panelists' task was to rank the samples according to the intensity of the perceived cat urine smell. Samples were coded and offered in randomized order as described above. The first ranking test was performed in duplicates with a gap of a few weeks between the sessions. Ten panelists participated in the first session and twelve panel members joined the second session. *Commercial Organic Cat Litter 1* as well as cat litter types containing 5 wt% xanthan gum, 2.5 wt% xanthan gum and 2.5 wt% Cationamyl 9854 or 10 wt% guar gum and 5 wt% carob gum were analyzed.

During the second pilot scale pelleting test two cat litter batches containing either fumed silicon oxide or activated carbon had been produced to further improve the odour absorption. In the following ranking test, *Commercial Organic Cat Litter 1* and cat litter containing 5 wt% xanthan gum and 1 wt% Cationamyl 9865 were compared to cat litter containing either 5 wt% xanthan gum, 1 wt% Cationamyl 9865 and 1 wt% fumed silicon oxide or 5 wt% xanthan gum, 1 wt% Cationamyl 9865 and 1 wt% activated carbon. Eleven panelists participated in this ranking test.

Furthermore, the odour absorption depending on the xanthan gum concentration was examined. In addition to that, a possible contribution of cationic starch on the reduction of unpleasant smell was investigated. Therefore cat litter samples containing 7 wt% xanthan gum, 5 wt% xanthan gum, 5 wt% xanthan gum and 1 wt% Cationamyl 9865 as well as 3 wt% xanthan gum and 1 wt% Cationamyl 9865 were analyzed. Thirteen panelists participated in this ranking test.

The aim of the last ranking test was to compare commercially available organic and inorganic cat litter types to those cat litter samples that showed the most promising results in the previous experiments. The analyzed samples were *Commercial Organic Cat Litter 1*, *Commercial Inorganic Cat Litter 1*, cat litter containing 5 wt% xanthan gum and cat litter containing 5 wt% xanthan gum and 1 wt% Cationamyl 9865. Twelve panelists took part in the ranking test.

Statistical evaluation of all ranking tests was performed by Friedman analysis. Panelists were not allowed to assign half ranks. With k samples, integer rank positions from 1 to k were possible. The number of panelists was n. The sum of

the squared rank sums R of all samples k, was determined according to formula 3.3. [13, 34]

$$R = \sum_{j=1}^{k} \left(\sum_{i=1}^{n} R_{i,j} \right)^2 \tag{3.3}$$

The test statistics, Friedman's T, was calculated using formula 3.4 [13, 34].

$$T = \frac{12}{n\,k\;(k+1)}\,R - 3\,n\;(k+1) \tag{3.4}$$

The probability p of T having a certain value was calculated using a χ^2 distribution by applying the null hypothesis of no difference between the samples at a significance level of 95 %. The number of degrees of freedom F was determined according to formula 3.5. [13,34] Afterwards the Microsoft Excel function *CHIVERT(Friedman's T; Degrees of Freedom)* could be applied to calculate p [13].

$$F = k - 1 \tag{3.5}$$

If p is smaller than the α error, a statistically significant difference between the samples is observed. The α error is a measure for the statistical uncertainty of the test. Its value is 0.05 at a significance level of 95 %. In case p is smaller than 0.05 the statistical certainty of a significant difference between the tested samples is larger than 95 %. [13, 34] The statistical certainty s can be written as follows:

$$s = 1 - p \tag{3.6}$$

To determine which samples differ significantly from each other Tukey's honestly significant difference (HSD) was calculated using formula 3.7. $q_{\alpha,k,\infty}$ is a tabulated value with α being the α error of 0.05 at a significance level of 95 % and k being the number of samples. For four samples $q_{\alpha,k,\infty}$ equals 3.63. Tukey's HSD can be applied independent of the overall statistical significance of the ranking test. [34]

$$HSD = q_{\alpha,k,\infty} \sqrt{\frac{n\,k\;(k+1)}{12}} \tag{3.7}$$

If the HSD is smaller than the difference of the rank sums of two samples, these two samples can be considered as significantly different [34].

4 Results and Discussion

4.1 Laboratory Scale Pelleting Tests

To understand how different additives react in combination with spruce sawdust, laboratory scale pelleting experiments were preformed using a mincer. At first, additives were tested individually. Later also blends of two additives with different concentrations were used. The obtained pellets differed significantly in length and texture. The variations were mainly depending on the type and the concentration of the additives. The results are summarized in figures 4.1 and 4.2.

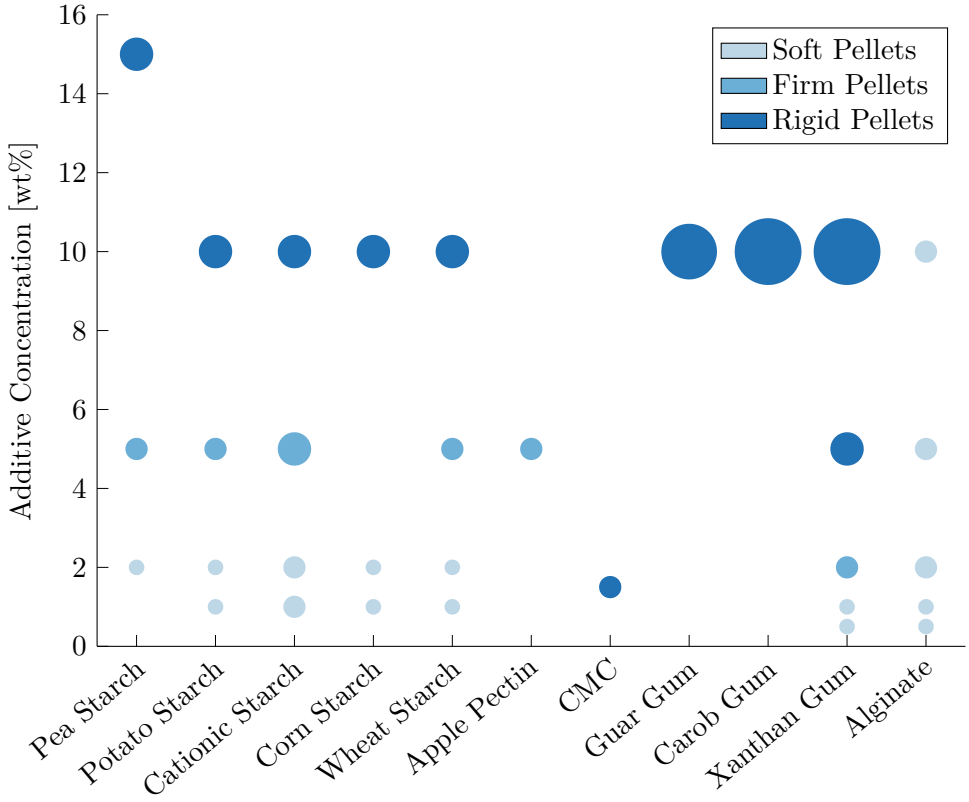

Figure 4.1: Pellets produced in the mincer using different additive types. The size of the spots represents the length of the pellets: 7 mm, 10 mm, 15 mm, 25 mm and 30 mm. The intensity of the colour represents the rigidity of the pellets. CMC = Carboxymethylcellulose.

Pellets containing one of the five different starch types showed very similar results. Concentrations higher or equal to 10 wt% resulted in very rigid particles that could not be squashed with the fingers. These samples never exceeded a pellet length of 15 mm. Generally, it was observed that the pellet length increased with increasing amounts of starch.

Apple pectin and most other pectin types preferably gel at low pH values up to a pH of approximately 3 [56]. However, the pH value of the sawdust, water and apple pectin mixture was 6 to 7[1]. Therefore it was not surprising that the pellets were only 10 mm long. Their texture was firm but not rigid.

1.5 wt% of CMC were sufficient to produce rigid particles. However, with a length of 10 mm the pellets were still considered to be short.

Guar gum and carob gum were not considered as additives in the final product as they are already part of other commercially available products. The experiments carried out helped to understand their properties. Guar gum and carob gum swelled using cold water. Therefore it was easier to press them through the mincer. The length of the pellets was 25 mm and 30 mm respectively, which is far longer than the length of the pellets produced earlier. After drying, the pellets were rigid and could not be squashed with the fingers.

The same behaviour was found with xanthan gum. It swelled after being contacted with cold water. Pellets containing 5 wt% and 10 wt% of xanthan gum were rigid. Using 2 wt% of xanthan gum resulted in firm pellets. Additive concentrations below this value led to soft pellets which could be easily squashed with the fingers.

All pellets produced with alginate were short and soft. Therefore this additive is not further investigated.

The first investigated additive blend was a 50:50 mixture of carrageen and carob gum. However, the pellets were soft and short with a length of 10 mm. Therefore this combination was not investigated further.

Guar gum and carob gum together acted well. Blends with one half, two thirds or five sixths of guar gum showed similar results considering the pellet length and texture. However, due to the above mentioned reasons, guar gum and carob gum were not considered for the final process.

The mixtures of xanthan gum and carob gum showed interesting properties. The total additive concentration of 5 wt% was kept constant throughout all experiments. Relative xanthan gum concentrations of 90 % or 83.33 % resulted in rigid pellets. When the relative carob gum concentration increased the pellet texture got softer. Therefore carob gum seems to weaken the pellets and should not be used in combination with xanthan gum. As the addition of 5 wt% xanthan gum led to rigid particles, there is no need to blend it with any other additive.

Mallow shred used in combination with cationic starch did not result in rigid particles. Even though 30 wt% and 35 wt% of this blend were added to the sawdust, both samples showed firm or even soft pellets. Therefore this combination was not further investigated.

[1] The pH value was determined using litmus paper.

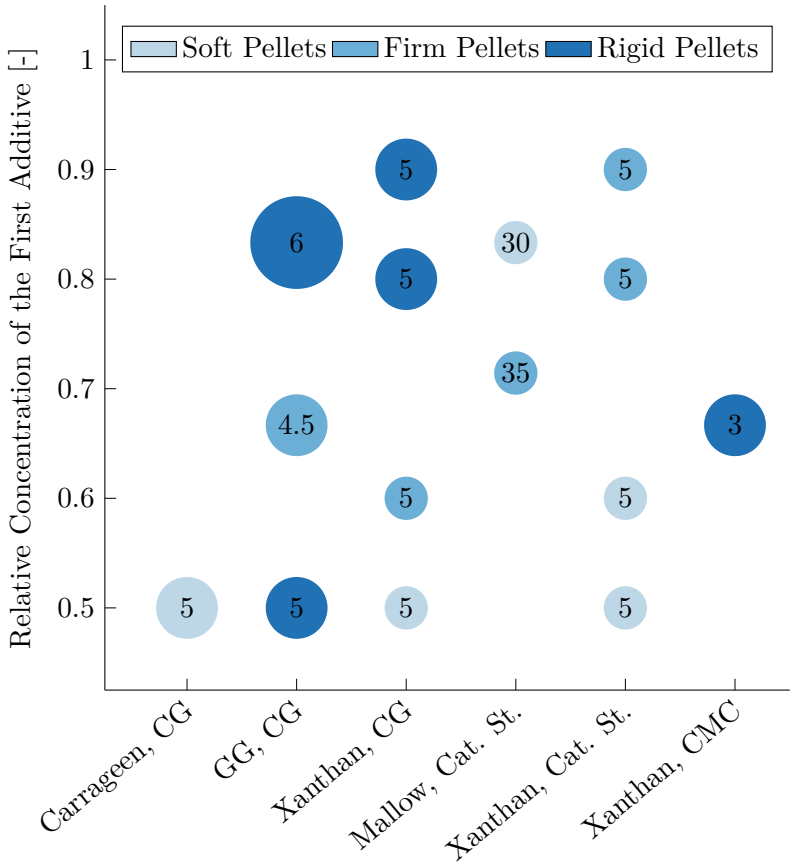

Figure 4.2: Pellets produced in the mincer containing blends of two additives. The total additive concentration in wt% is shown by the number in the center of the spots. The size of the spots represents the length of the pellets: 7 mm, 10 mm and 15 mm. The intensity of the colour represents the rigidity of the pellets. CG = Carob Gum, GG = Guar Gum, Cat. St. = Cationic Starch, CMC = Carboxymethylcellulose.

Xanthan gum mixed with cationic starch resulted in firm or soft pellets, depending on the relative concentration of xanthan gum. Larger relative amounts of xanthan gum led to firm pellets. Increasing the relative concentration of cationic starch made the pellets' texture soft. As cationic starch was expected to have antimicrobial effects, this additive was further investigated. Therefore different types of cationic starch with other swelling properties were tested in the following experiments.

Finally, a blend consisting of two thirds xanthan gum and one third carboxymethylcellulose was investigated. The total additive amount was 3 wt%. The resulting pellets were rigid and had a length of 10 mm. Nevertheless, using a slightly higher amount of xanthan gum only (approximately 5 wt%) is more recommendable than a combination with carboxymethylcellulose. This is due to the high price of CMC

and the fact that no significant improvement could be achieved by its addition.

Xanthan gum is the most promising additive in this large selection of substances. None of the mixtures caused improved pellet properties compared to pellets containing xanthan gum only. However, the antimicrobial action of cationic starch should be further investigated to use it as a second additive.

4.2 Water Uptake Capacity of Cat Litter

4.2.1 Commercially Available Products

The water uptake capacity of two inorganic and three biologically degradable cat litter types was determined. The results were used as reference values for self-produced cat litter samples. There were high-price as well as low-price products among the inorganic and the organic samples. The results are summarized in table 4.1. Due to the large density differences of the materials, inorganic cat litter samples could not be directly compared with organic cat litter types.

The water uptake capacity of *Commercial Inorganic Cat Litter 1* was higher than that of *Commercial Inorganic Cat Litter 2*. All four samples of *Commercial Inorganic Cat Litter 1* showed that a lower mass of this litter type was needed to take up 50 mL of water. The deviation between clumps produced by the titrating burette was 2.9 g for *Commercial Inorganic Cat Litter 1* and 4.91 g for *Commercial Inorganic Cat Litter 2*. The scatter range of the samples prepared with the graduated cylinder was wider than that of the samples produced with the burette. The deviation between the clumps prepared using the measuring cylinder ranged from 6.6 g for *Commercial Inorganic Cat Litter 1* to 15.18 g for *Commercial Inorganic Cat Litter 2*.

Commercial Organic Cat Litter 1 formed stable clumps. The particles had a maximum dimension of approximately 5 mm. Their shape resembled polymer granulate or corn grit. Clumps prepared using the graduated cylinder contained approximately 35 g of dry litter material. For clumps prepared with the burette, 26 g of dry cat litter are consumed on average.

Commercial Organic Cat Litter 2 did not form defined clumps. In addition to that, the morphology of this litter type resembled that of heating pellets and differed significantly from that of all other samples. The pellets were of cylindrical shape with an average length of 3 cm and a smooth surface. After contacting the pellets with water, it took some time until the pellets started to swell. However, most of the water was not absorbed. It was found at the bottom of the plastic container. As no clumps were formed, no evaluation considering the water uptake capacity could be carried out.

Commercial Organic Cat Litter 3 is a granular material made of spheroidal particles with an average diameter of 3 mm. The method using the graduated cylinder required between 35 g and 38 g of dry litter material per clump. In case the burette was used, between 44 g and 50 g of dry litter were consumed. In three out of four

cases, less *Commercial Organic Cat Litter 1* than *Commercial Organic Cat Litter 3* was needed to absorb 50 mL of water. This means that *Commercial Organic Cat Litter 1* was more economical than *Commercial Organic Cat Litter 3*.

In general, all clumps showed a similar size, independent of their basic material. *Commercial Inorganic Cat Litter 1* had the highest water uptake capacity of the inorganic samples, whereas *Commercial Organic Cat Litter 1* achieved the highest value among the organic litter types. Besides the biodegradability of organic cat litter, the much lower density of the organic material can be advantageous for the user. On the other hand, the lighter particles might tend to stick to the fur or the paws of the cat leading to a spreading of the litter outside the cat toilet.

Table 4.1: Water uptake capacity of commercially available organic and inorganic cat litter types. The mass of dry litter m_{litter} was calculated by subtracting the mass of deionized water, corresponding to 50 mL (49.852 g at 25 °C), from the mass of the clump m_{clump}.

	Method	Time [s]	m_{clump} [g]	m_{litter} [g]
Commercial Inorganic Cat Litter 1	Cylinder	15	136.70	86.85
		14	143.30	93.45
	Burette	30	120.00	70.15
		25	122.90	73.05
Commercial Inorganic Cat Litter 2	Cylinder	15	160.20	110.35
		17	145.02	95.17
	Burette	25	143.24	93.39
		25	138.33	88.48
Commercial Organic Cat Litter 1	Cylinder	15	85.98	36.13
		15	85.11	35.26
	Burette	25	76.09	26.24
		25	77.36	27.51
Commercial Organic Cat Litter 2	Cylinder	15	-	-
		15	-	-
	Burette	25	-	-
		25	-	-
Commercial Organic Cat Litter 3	Cylinder	15	85.03	35.18
		15	87.84	37.99
	Burette	25	93.80	43.95
		26	99.90	50.05

4.2.2 Initial Pilot Scale Pelleting Products

The water uptake capacity of cat litter types produced during the first pilot scale pelleting experiment was determined. The results are listed in table 4.2. The

reference sample containing only spruce sawdust did not form stable clumps. All four samples broke into pieces when they were withdrawn from the plastic box using a sieve scoop.

Batch 2 contained 2 wt% of xanthan gum. The formed clumps were hardly stable. The sieve scoop had to be handled very carefully to avoid breakage of the clumps during withdrawal from the plastic container. Light finger pressure was enough to break the cat litter nuggets. On average, 42.3 g of dry litter were necessary to absorb 50 mL of water.

At a xanthan concentration of 5 wt% robust clumps were formed. Their shape remained stable when they were taken out of the box and even when high finger pressure was applied. An average cat litter mass of 35.7 g was consumed to absorb the sample volume.

Batch 4 contained 4.5 wt% xanthan gum and 0.5 wt% carob gum. In a previously performed side experiment it was demonstrated that these two additives show a synergistic effect: an aqueous solution of 1 %(w/v) xanthan gum and 0.5 %(w/v) carob gum exhibited a much higher viscosity than a 2 %(w/v) xanthan gum solution. As carob gum is more expensive than xanthan gum, the mixing ratio was reduced in the pelleting experiment. During the water uptake capacity test no improvement of the shape stability or the water uptake ability could be noticed compared to the 5 wt% xanthan gum cat litter nuggets. On the contrary, two out of four samples could not absorb the water fast enough to prevent the applied water from streaming down at the inner wall of the plastic container. Hardly any clumps were formed for these two samples. Therefore they could not be considered for evaluation. The respective samples were the first one produced by the graduated cylinder (−0.12 g litter mass) and the third one produced with the burette (5.65 g litter mass). The mean dry cat litter mass of the two remaining clumps was 23.6 g. For the reasons mentioned, the possible synergistic effect of xanthan gum and carob gum was not developed further.

Batches 5 to 7 (2.5 wt% xanthan gum and 2.5 wt% potato starch, Cationamyl 9854 or Cationamyl 9865) exhibited similar clumping properties. Withdrawing the nuggets from the litter box using the sieve scoop could be performed without any problems. Despite high finger pressure was applied, the nuggets' shape was stable. However, batch 5 containing potato starch was slightly less robust than the other two litter types. The mean masses of dry cat litter necessary to absorb 50 mL of water were 36.4 g for batch 5, 46.1 g for batch 6 and 38.4 g for batch 7.

Batch 8 contained 10 wt% of guar gum and 5 wt% of carob gum. This composition was chosen to qualitatively and quantitatively imitate the additives used in *Commercial Organic Cat Litter 1* based on the product description given in the referring patent [40]. Good clumping ability and robust shapes were detected. 50 mL of water could be absorbed by 21.8 g of dry litter material. However, due to the high amount of additives such a product would not be economically viable. Considering all factors, batch 3 containing 5 wt% of xanthan gum was the most promising litter type of the first pilot scale pelleting experiment.

Table 4.2: Water uptake capacity of cat litter types produced during the initial pilot scale pelleting test. The additive amount is given in wt%. The mass of dry litter m_{litter} was calculated by subtracting the mass of deionized water, corresponding to 50 mL (49.852 g at 25 °C), from the mass of the clump m_{clump}.

	Method	Time [s]	m_{clump} [g]	m_{litter} [g]
Reference	Cylinder	15	-	-
		15	-	-
	Burette	25	-	-
		25	-	-
2 % Xanthan	Cylinder	15	97.60	47.75
		16	86.60	36.75
	Burette	25	90.53	40.68
		27	93.81	43.96
5 % Xanthan	Cylinder	15	88.49	38.64
		18	82.74	32.89
	Burette	28	91.21	41.36
		28	79.74	29.89
4.5 % Xanthan 0.5 % Carob Gum	Cylinder	16	49.73	−0.12
		17	76.79	26.94
	Burette	28	55.50	5.65
		27	70.16	20.31
2.5 % Xanthan 2.5 % P. Starch[1]	Cylinder	16	83.19	33.34
		15	95.43	45.58
	Burette	25	86.19	36.34
		27	80.16	30.31
2.5 % Xanthan 2.5 % Cat2[2]	Cylinder	15	92.37	42.52
		15	92.30	42.45
	Burette	26	101.90	52.05
		25	97.27	47.42
2.5 % Xanthan 2.5 % Cat1[3]	Cylinder	13	101.07	51.22
		15	77.97	27.94
	Burette	24	83.68	33.83
		23	90.29	40.44
10 % Guar Gum 5 % Carob Gum	Cylinder	16	69.46	19.61
		13	74.17	24.32
	Burette	25	70.68	20.83
		24	72.28	22.43

[1] Potato Starch [2] Cationamyl 9865 [3] Cationamyl 9854

4.2.3 Subsequent Pilot Scale Pelleting Products

The water uptake capacity of cat litter types produced during the second pilot scale pelleting experiment was determined. Clumps were formed by all litter types except the reference sample consisting of spruce sawdust only. The results of the water uptake capacity test are summarized in table 4.3.

Careful sieve scoop handling was necessary to withdraw the clumps formed by batch 2, containing 3 wt% xanthan gum and 1 wt% Cationamyl 9865. Already at light finger pressure, the clumps broke apart. The average dry litter mass necessary to absorb 50 mL of water was 35.2 g.

An additive concentration of 4 wt% xanthan gum and 1 wt% Cationamyl 9865 led to clumps with increased shape stability. However, the water uptake rate was lower resulting in water streaming along the inner wall of the box. As only a small unknown volume of water was absorbed in the clumps they could not be used for water uptake capacity evaluation.

Clumps of high stability were formed in case of batch 4 litter containing 5 wt% xanthan gum and 1 wt% Cationamyl 9865. No breakage occurred when the nuggets were taken out of the plastic box and when high finger pressure was applied. The last sample of this litter type could not be considered for evaluation of the water uptake capacity as some water streamed down the inner container wall. The average dry litter mass of 30.25 g was only calculated from the remaining three nuggets.

An additive concentration of 7 wt% xanthan gum resulted in extremely stable cat litter clumps. Handling with the sieve scoop could be performed without any breakage. Applying high finger pressure did not lead to shape instabilities. The mean dry litter mass necessary to absorb the applied water volume was 34.7 g.

In contrast to the previous batches, the last two litter types contained three different additives. Batch 6 included 5 wt% xanthan gum, 1 wt% Cationamyl 9865 and 1 wt% activated carbon. Activated carbon did not influence the clumping properties, but it was thought to increase the odour absorption ability of the litter. Batch 6 resembled batch 4 (5 wt% xanthan gum and 1 wt% Cationamyl 9865). The litter nuggets were resistant to pressure and could be handled with the sieve scoop without breakage. One sample could not be considered for the water uptake capacity evaluation as some water streamed down the inner container wall. The mean dry litter mass of 31.6 g was calculated from the three remaining samples.

Batch 7 contained 1 wt% of fumed SiO_2 particles instead of activated carbon. This additive did not affect the clumping ability, but was also expected to improve odour absorption properties. The litter nuggets did not differ significantly from those of batches 4 and 6. The mean dry cat litter mass necessary for water absorption was 40.9 g.

None of the litter types exhibited large deviations between clumps formed by the graduated cylinder or the burette. Batch 4 showed the best water uptake capacity with 30.25 g per 50 mL of water.

Table 4.3: Water uptake capacity of cat litter types produced during the second pilot scale pelleting test. The additive amount is given in wt%. The mass of dry litter m_{litter} was calculated by subtracting the mass of deionized water, corresponding to 50 mL (49.852 g at 25 °C), from the mass of the clump m_{clump}.

	Method	Time [s]	m_{clump} [g]	m_{litter} [g]
Reference	Cylinder	15	-	-
		15	-	-
	Burette	25	-	-
		25	-	-
3 % Xanthan 1 % Cat2[1]	Cylinder	16	85.29	35.44
		17	88.64	38.79
	Burette	20	87.65	37.80
		21	78.74	28.89
4 % Xanthan 1 % Cat2	Cylinder	15	40.30	−9.55
		16	56.75	6.90
	Burette	20	60.45	10.60
		20	67.92	18.07
5 % Xanthan 1 % Cat2	Cylinder	16	73.77	23.92
		18	87.04	37.19
	Burette	20	79.49	29.64
		19	59.31	9.46
7 % Xanthan	Cylinder	16	84.05	34.20
		18	79.85	30.00
	Burette	21	87.27	37.42
		21	87.16	37.31
5 % Xanthan 1 % Cat2 1 % AC[2]	Cylinder	17	87.02	37.17
		15	86.08	36.23
	Burette	23	71.11	21.26
		24	53.83	3.98
5 % Xanthan 1 % Cat2 1 % Silica[3]	Cylinder	15	85.62	35.77
		13	81.33	31.48
	Burette	19	105.29	55.44
		21	90.84	40.99

[1] Cationamyl 9865 [2] Activated Carbon [3] Fumed SiO_2 particles

4.2.4 Industrial Scale Pelleting Products

The water uptake capacity of cat litter types produced during the industrial scale pelleting experiment was determined. All batches except the reference litter formed clumps. The results of the water uptake capacity test are listed in table 4.4.

Table 4.4: Water uptake capacity of cat litter types produced during the industrial scale pelleting test. Additionally, all batches including the reference contain 0.5 wt% of pelleting starch. The additive amount is given in wt%. The mass of dry litter m_{litter} was calculated by subtracting the mass of deionized water, corresponding to 50 mL (49.852 g at 25 °C), from the mass of the clump m_{clump}.

	Method	Time [s]	m_{clump} [g]	m_{litter} [g]
Reference	Cylinder	16	-	-
		16	-	-
	Burette	26	-	-
		22	-	-
3 % Xanthan	Cylinder	18	65.17	15.32
		15	84.47	34.62
	Burette	27	82.27	32.42
		26	88.50	38.65
4 % Xanthan	Cylinder	18	82.59	32.74
		12	78.34	28.49
	Burette	19	71.01	21.16
		23	92.67	42.82
5 % Xanthan	Cylinder	17	83.85	34.00
		13	86.54	36.69
	Burette	18	82.61	32.76
		19	91.52	41.67
3 % Xanthan 1 % Sobocat	Cylinder	15	85.19	35.34
		16	81.59	31.74
	Burette	23	84.48	34.63
		24	84.34	34.49
4 % Xanthan 1 % Sobocat	Cylinder	14	91.50	41.65
		15	85.94	36.09
	Burette	21	87.01	37.16
		24	76.87	27.02
5 % Xanthan 1 % Sobocat	Cylinder	16	87.24	37.39
		14	81.73	31.88
	Burette	22	85.24	35.39
		20	85.34	35.49

Batch 2 contained 3 wt% of xanthan gum. Clumps were hardly stable when taking them out of the box with the sieve scoop or when applying light finger pressure. The first sample was not considered for the evaluation of the water uptake capacity as some water streamed down the inner container wall and was therefore not included

in the clump. The average dry mass of cat litter needed for taking up 50 mL of water was calculated from the remaining three samples, giving a value of 35.23 g.

4 wt% of xanthan gum led to stable clumps that resisted handling with the sieve scoop and also higher finger pressure. The mean dry cat litter mass for absorbing the applied liquid was 31.3 g.

Batch 4 included 5 wt% of xanthan gum. The formed cat litter nuggets were as stable as the ones produced from batch 3. The mean dry litter mass needed for absorption of 50 mL water was 36.3 g.

The batches 5 to 7 can be seen as equivalent to batches 2 to 4, except that each of them contained an additional 1 wt% of cationic starch type Sobocat. The properties regarding shape stability and clumping ability were equal to those of their counterpart batches. The mean dry litter masses necessary for complete water absorption were 34.1 g in case of batch 5 (3 wt% xanthan gum and 1 wt% Sobocat), 35.5 g in case of batch 6 (4 wt% xanthan gum and 1 wt% Sobocat) and 35.0 g in case of batch 7 (5 wt% xanthan gum and 1 wt% Sobocat).

None of the cat litter types showed any significant difference between clumps prepared with the graduated cylinder or the burette. The most promising results were exhibited by batch 3.

4.2.5 Crushed Cat Litter Pellets

Crushed 2 wt% xanthan gum cat litter did not form stable clumps during the water uptake capacity test. Even though most of the applied water was absorbed by the litter material, the nuggets broke apart when they were handled with the sieve scoop. Therefore no evaluation according to the water uptake capacity could be performed.

The poor clumping ability could on the one hand result from the low additive concentration of 2 wt% xanthan gum. On the other hand, the average particle size was larger than 5 mm. The combination of these two effects resulted in unstable pellets. Using more additive and decreasing the average particle size would solve the problem.

The investigated commercially available cat litter types formed clumps showing a similar size, independent of their basic material. However, clumps from inorganic cat litter brands were twice or three times as heavy as clumps from organic ones. This is due to the higher density of the basic material bentonite. Cat litter types containing at least 4 wt% of xanthan gum formed stable clumps and could be removed from the cat litter box without breakage. Batches containing 2 wt% and 3 wt% of xanthan gum were hardly stable. Considering both, clump stability and water uptake capacity, the litter type containing 5 wt% of xanthan gum was the most promising batch produced during the first pilot scale pelleting experiment. On average 35.7 g of this litter were necessary to absorb 50 mL of deionized water. During the subsequent pilot scale pelleting experiment the litter type containing 5 wt% of xanthan gum and 1 wt% Cationamyl 9865 showed the best water uptake

capacity with 30.25 g of litter necessary to take up 50 mL of deionized water. In the industrial scale pelleting experiment the highest water uptake capacity was determined for batch 3, containing 4 wt% of xanthan gum. In this case 31.3 g of dry litter were necessary to absorb 50 mL of deionized water. In general, the average masses needed to take up the test volume of 50 mL were in the same range as the ones of the investigated commercially available organic cat litter types.

4.3 Comminution via Crushing, Cutting Mill and Ball Mill

Crushing, cutting and milling were investigated for generation of the desired particle size and shape. Figure 4.3 shows the visual assessment of crushed 2 wt% xanthan gum cat litter, *Commercial Organic Cat Litter 1* and *Commercial Organic Cat Litter 3*. *Commercial Organic Cat Litter 3* consisted of smaller particles than the other two litter materials. The maximum particle dimension of *Commercial Organic Cat Litter 1* was in the range of 5 mm. This approximation was based on the fact that the samples were placed on commercial squared paper with a box width of 5 mm. The majority of the crushed 2 wt% xanthan gum cat litter particles was larger than one box and therefore larger than 5 mm.

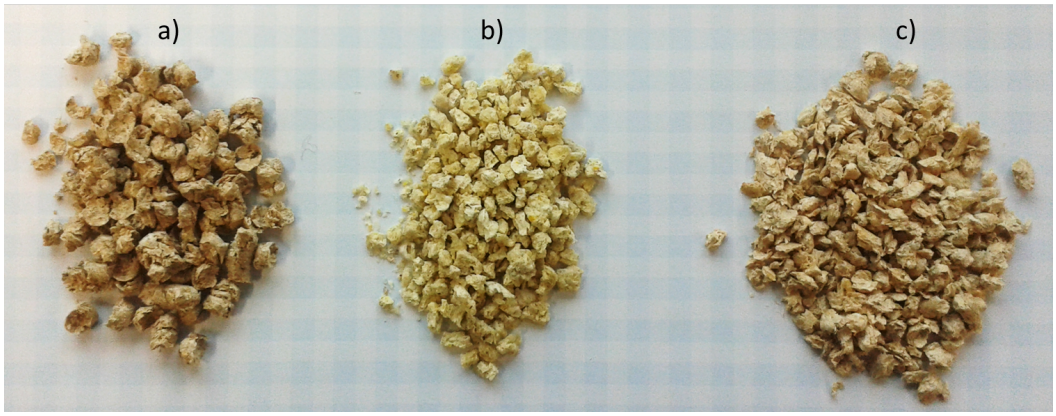

Figure 4.3: Visual comparison of a) crushed 2 wt% xanthan gum cat litter, b) *Commercial Organic Cat Litter 3* (no further treatment) and c) *Commercial Organic Cat Litter 1* (no further treatment).

Milling using a ball mill did not fragment the pellets in the desired way, but produced a powder. After 1 min of milling, small, smooth pellet fragments surrounded by fine sawdust were found in the milling cup. The result of this experiment can be seen in figure 4.4. None of the particles showed the desired textured surface present at all samples visible in figure 4.3. No additional sieve analysis was performed as the visual assessment was enough to not further pursue this comminution method.

The visual assessment showed that crushing generated the desired textured surface. However, the particle size was higher than 5 mm. This might be one reason

Figure 4.4: 57.8 g of 7 wt% xanthan gum cat litter after milling for 1 min in a 200 mL milling cup with milling balls (both made from agate stone).

for the bad clumping ability reported in subsection 4.2.5. Milling was not an appropriate comminution method as neither the resulting particle size nor the surface structure were suitable for the application as cat litter.

4.3.1 Sieve Analysis

The particle size distributions of *Commercial Organic Cat Litter 1*, crushed 2 wt% xanthan gum cat litter, cut 5 wt% xanthan gum cat litter and cut 7 wt% xanthan gum cat litter were determined using a sieve stack. The results of the sieve analysis are listed in table 4.5. The resulting density distributions and cumulative representations of all particle size distributions are shown in figures 4.5 and 4.6.

53 wt% of the total mass of *Commercial Organic Cat Litter 1* were found on the top sieve. This means that more than half of the *Commercial Organic Cat Litter 1* particles were larger than 3.15 mm. In the density distribution this is represented by the largest peak at the far right side of the diagram. 13 wt% of *Commercial Organic Cat Litter 1* show a particle size between 2.8 mm and 3.15 mm. 18 wt% could be found between 2 mm and 2.8 mm. The remaining fractions had a relative amount of up to 5 % each. The density distribution showed a flattening curve in regions of decreasing mesh size and particle size respectively. The same properties were seen in the cumulative distribution in figure 4.6. The first five points in the lower mesh size region were close together and the curve was rising slowly. Particles up to a size of 2 mm made up less than 20 % of the total sample mass. At a mesh

Table 4.5: Mesh sizes, mass of the oversize material $m_{oversize}$ and fractions of over- and undersize material, $f_{oversize}$ and $f_{undersize}$, of *Commercial Organic Cat Litter 1*, crushed 2 wt%, cut 5 wt% and cut 7 wt% xanthan gum cat litter.

	Mesh Size [mm]	$m_{oversize}$ [g]	$f_{oversize}$ [wt%]	$f_{undersize}$ [wt%]
Commercial Organic Cat Litter 1	3.15	51.7	53	47
	2.8	12.9	13	34
	2	17.4	18	16
	1.4	4.8	5	11
	1	2.9	3	8
	0.8	3.1	3	5
	0.5	1.7	2	3
	0.25	2.1	2	1
	0	1.1	1	2
2 % Xanthan Crushed	3.15	72.8	71	29
	2.8	5.4	5	23
	2	6.8	7	17
	1.4	7.4	7	9
	1	3.5	3	6
	0.8	1.5	1	5
	0.5	2.2	2	2
	0.25	1.4	1	1
	0	1.0	1	1
5 % Xanthan Cutting Mill	3.15	13.7	14	86
	2.8	5.6	6	80
	2	18.4	19	60
	1.4	17.6	19	42
	1	14.7	15	26
	0.8	6.5	7	19
	0.5	9.8	10	9
	0.25	6.5	7	2
	0	2.1	2	7
7 % Xanthan Cutting Mill	4	3.8	2	98
	2	43.7	26	72
	1.6	21.1	12	60
	0	102.1	60	0

size of 2 mm the curve started to rise fast. This indicated that the majority of the particles was larger than 2 mm. Generally, *Commercial Organic Cat Litter 1* had a narrow particle size distribution with 84 wt% of the particles being larger than 2 mm and 53 % being larger than 3.15 mm.

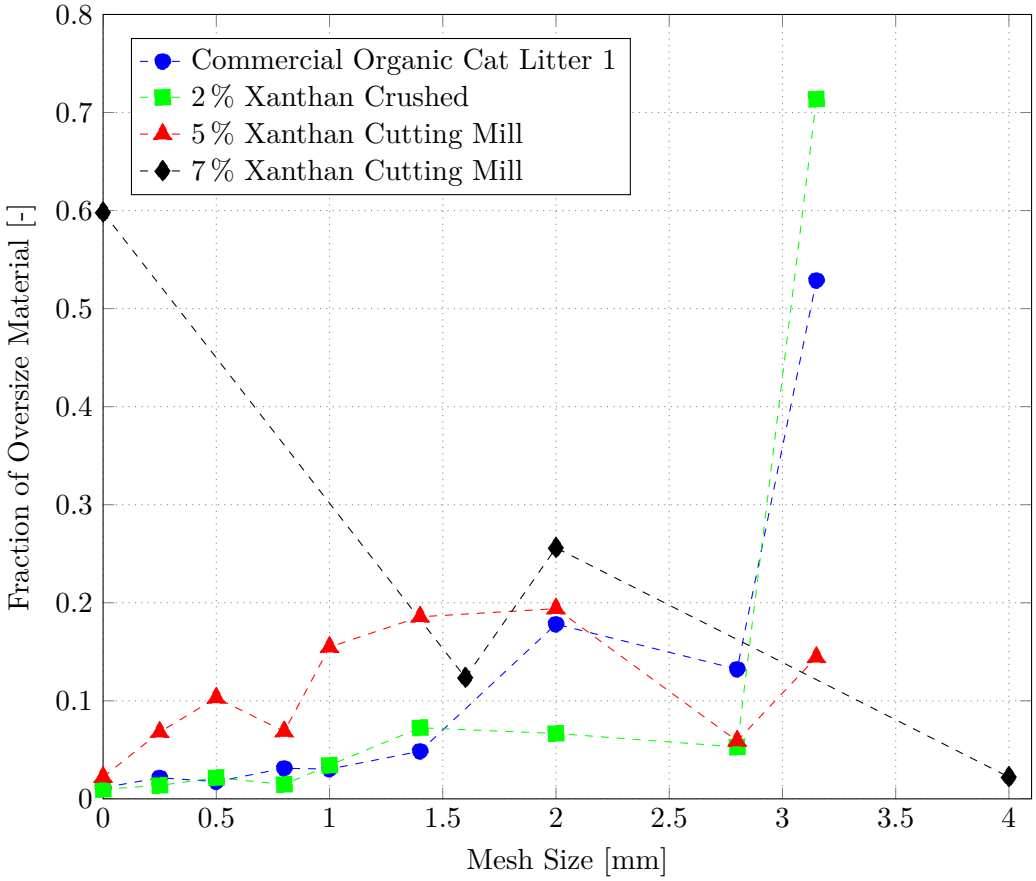

Figure 4.5: Density function of the particle size distribution of *Commercial Organic Cat Litter 1*, 2 wt% xanthan gum cat litter (crushed), 5 wt% and 7 wt% xanthan gum cat litter (cut).

Crushed 2 wt% xanthan gum cat litter had an even narrower particle size distribution than *Commercial Organic Cat Litter 1*. 71 wt% of the total mass of 102 g were larger than 3.15 mm. This result also confirmed the observation made during the visual assessment of the sample. Each of the other fractions held 7 wt% of the total mass or less. Again the density distribution showed the highest peak at the far right side (cf. figure 4.5). The cumulative distribution curve increased slowly in the small mesh size region and got very steep at the largest mesh size (cf. figure 4.6).

The particle size distribution of cut 5 wt% xanthan gum cat litter was broad with the majority of particles ranging from 1 mm to 2 mm. Such properties can be disadvantageous as small particles tend to stick to the cats' fur or paws. In addition to that, larger amounts of dust can occur during refilling of the cat toilet. Figure 4.5 confirms the broad particle size distribution as none of the fractions made up more than 20 wt% of the total sample mass. The cumulative distribution showed

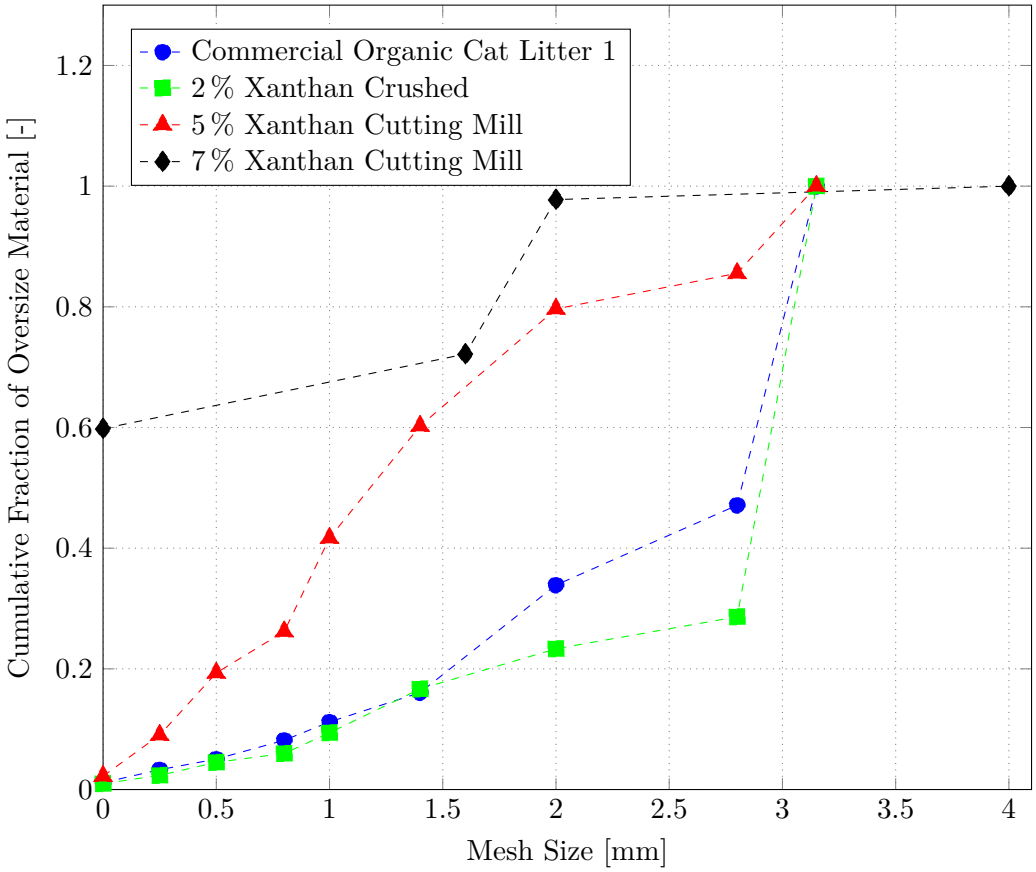

Figure 4.6: Cumulative density function of the particle size distribution of *Commercial Organic Cat Litter 1*, 2 wt% xanthan gum cat litter (crushed), 5 wt% and 7 wt% xanthan gum cat litter (cut).

an almost constant slope stating that there was no accumulation of particles at a certain sieve (cf. figure 4.6). The desired PSD of the final product is narrow, preferably with the majority of particles having a size of approximately 5 mm. This might be achieved by a cutting mill with an adjustable number of knives or cutting velocity.

The analysis of the 7 wt% xanthan gum cat litter showed that 60 wt% of the total sample mass were smaller than 1.6 mm (cf. table 4.5). Due to a lack of sieves, no further fractions between a mesh size of 0 and 1.6 mm could be derived. 60 wt% of the previous sample (5 wt% xanthan gum cat litter) also consisted of particles smaller than 2 mm. Even though the curves had different shapes, the PSD for the low mesh sizes was similar. Particles between 2 mm and 4 mm made up 26 wt% of the mass of the 7 wt% xanthan gum litter. In case of the 5 wt% xanthan gum litter material 39 wt% could be found in this region. However, no sieve with a mesh size of 4 mm was used here. Therefore all larger particles were located on the 3.15 mm

sieve. No further differentiation was possible. Generally, it needs to be considered that the particles were non-spherical and compressible. In such a case the sieving time as well as the particle orientation on the sieve have a huge impact on the result of the experiment.

The particle size distribution did not depend on the additive concentration. However, the method of comminution had a tremendous impact on the resulting grain size. It can be expected that the particle size distribution will remain the same for different litter formulations, as long as the same comminution method is used. None of the tested machines generated cat litter of the desired particle size distribution. Milling caused abrasion of the particles by the milling balls resulting in fine powder. Crushing and cutting led to the desired textured surface. However, crushing resulted in a particle size distribution with too many large particles as 71 % of the particles were larger than 3.15 mm. In combination with the low xanthan gum concentration of this sample (2 wt%) no stable clumps could be formed during the water uptake capacity test. Cutting resulted in a particle size distribution with too many small particles. This led to a reduced water uptake rate and caused problems such as some liquid flowing down the inner wall of the box during the water uptake capacity tests. Besides switching to other comminution techniques, it would also be possible to separate certain particle fractions from the total mass by e.g. sieving or air classification. A combination of a suitable comminution and separation technique will most likely yield the preferred particle size distribution.

4.4 Microbiological Experiments

4.4.1 Growth Behaviour at the Pellet Surface

E. coli, *Micrococcus ssp.*, *Bacillus subtilis* and *Aspergillus brasiliensis* were used as test organisms in the first experiment. All overnight cultures showed comparable levels of microbial growth. The optical density of the sterile control was 0. It could be assumed that each test tube contained only the inoculated species of bacteria or filamentous fungi. In figure 4.7 the number of colonies found on each resuspension agar plate can be seen. Table 4.6 shows the number of colony forming units (CFU) per mL of resuspension volume and the number of CFU per g of litter.

The drying chamber heated to 45 °C over night, a temperature at which *E. coli*, *Micrococcus ssp.* and *Bacillus subtilis* were not viable. No colonies were formed at the growth control agar plates and the agar plates containing the resuspended pellet supernatant. After 72 h of incubation the *Aspergillus brasiliensis* growth control agar plate was fully covered by a fungi layer. The resuspension agar plates contained between 53 and 115 colonies after incubation was ended (cf. Exp 1 in figure 4.7). Either 1 or 2 colonies were found in case of the 100-fold diluted pellet resuspension (cf. second section of table 4.6). The ratio of colonies found on the agar plates containing undiluted and diluted resuspension corresponded to the dilution factor. The reference litter sample, 5 wt% xanthan gum litter and cat

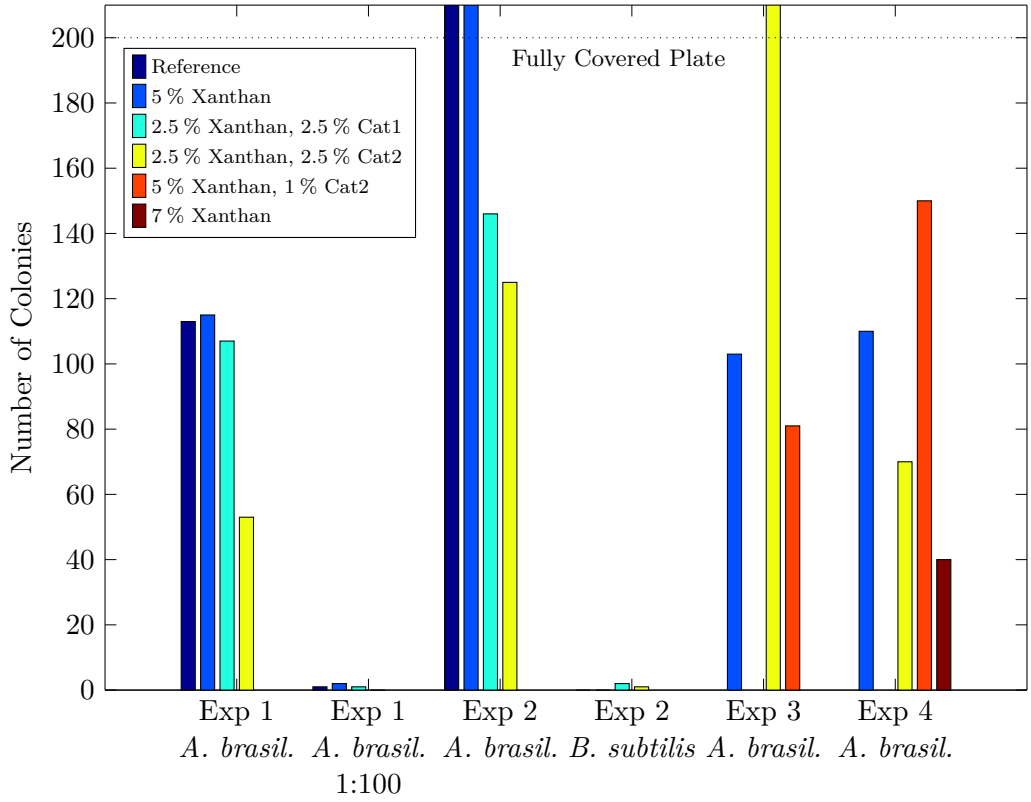

Figure 4.7: Colony numbers of 100 µL resuspension volume (undiluted or 100-fold diluted) of cat litter samples inoculated with *Aspergillus brasiliensis* or *Bacillus subtilis* plated on blood agar plates. The additive concentration of the cat litter material is given in wt%. Cat1 = Cationamyl 9854, Cat2 = Cationamyl 9865.

litter containing 2.5 wt% xanthan gum and 2.5 wt% Cationamyl 9854 showed similar values of CFU per mL (cf. first section of table 4.6). The respective numbers of CFU per g differed from each other. Litter containing 2.5 wt% xanthan gum and 2.5 wt% Cationamyl 9865 displayed the smallest number of CFU per mL as well as per g. The diluted samples showed CFU values of the same order of magnitude. However, the error of dilution was large here.

E. coli, *Bacillus subtilis* and *Aspergillus brasiliensis* were used as test organisms in the second experiment (Exp 2). Each ONC showed a similar optical density. No microbial growth could be detected in the sterile control of the ONC, as well as the sterile control of the growth control. Growth control agar plates of *E. coli* and *Bacillus subtilis* were fully covered by bacteria layers after 24 h. In the previous experiment it was observed that *Aspergillus brasiliensis* hyphae require at least 72 h to become visible. After 96 h of incubation a fungi layer was displayed on the *Aspergillus brasiliensis* growth control plate. Two *Bacillus subtilis* colonies grew on top of the resuspension agar plate of the 2.5 wt% xanthan gum, 2.5 wt% Cationamyl

Table 4.6: Pellet mass m_{pellet}, media volume for pellet resuspension V, dilution factor of the plated volume DF as well as the calculated colony forming units per mL of resuspension volume CFU/mL and per g of pellets CFU/g for all litter types and microorganisms showing colonies on the blood agar plates. The additive concentration of the litter is given in wt%, n.d. refers to not determinable.

Litter	Organism	m_{pellet} [g]	V [mL]	DF	CFU/mL	CFU/g
Reference	*A. brasil.*	9.36	60	1	1130	7244
5 % X[1]		15.08	150	1	1150	11 439
2.5 % X, 2.5 % C1[2]		18.83	75	1	1070	4262
2.5 % X, 2.5 % C2[3]		15.73	100	1	530	3369
Reference	*A. brasil.*	9.36	60	100	1000	6410
5 % X		15.08	150	100	2000	19 894
2.5 % X, 2.5 % C1		18.83	75	100	1000	3983
2.5 % X, 2.5 % C2		15.73	100	100	1000	6357
Reference	*A. brasil.*	11.89	50	1	n.d.	n.d.
5 % X		17.71	75	1	n.d.	n.d.
2.5 % X, 2.5 % C1		14.04	50	1	1460	5199
2.5 % X, 2.5 % C2		13.09	50	1	1250	4775
Reference	*B. subtilis*	12.98	50	1	0	0
5 % X		16.80	75	1	0	0
2.5 % X, 2.5 % C1		12.40	50	1	20	81
2.5 % X, 2.5 % C2		13.11	50	1	10	38
5 % X	*A. brasil.*	13.26	50	1	1030	3884
5 % X, 1 % C2		13.07	50	1	810	3099
2.5 % X, 2.5 % C2		15.58	50	1	n.d.	n.d.
7 % X	*A. brasil.*	14.92	50	1	400	1340
5 % X		16.35	50	1	1100	3364
5 % X, 1 % C2		18.82	50	1	1500	3985
2.5 % X, 2.5 % C2		19.03	50	1	700	1839

[1] Xanthan Gum [2] Cationamyl 9854 [3] Cationamyl 9865

9854 cat litter after 144 h of incubation (cf. Exp 2 in figure 4.7). One colony was displayed in case of the 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9865 sample. No *E. coli* colonies were formed. The growth conditions at the litter pellet surface were too harsh for bacteria. Most likely the water activity was too low. *Bacillus subtilis* can form spores under stress conditions like drought. This might have been the reason why few colonies grew at the agar plates. However, such a low number of CFU per mL as well as per g indicated that bacterial growth was not an issue for the application as cat litter (cf. fourth section of table 4.6). *Aspergillus brasiliensis* was more resistant to low water activities. After 72 h the agar plates containing the

resuspension aliquots of the reference as well as the 5 wt% xanthan gum litter were completely covered with a fungi layer. In figure 4.7 this was represented by bars exceeding the border of completely covered plates. Generally, it was not possible to distinguish individual colonies if more than 200 colonies were formed. As no exact colony number could be counted, the number of colony forming units could not be calculated. Cat litter containing 2.5 wt% xanthan gum combined with either 2.5 wt% Cationamyl 9854 or Cationamyl 9865 showed higher numbers of CFU than the respective samples in the previous experiment (cf. third section of table 4.6). Nevertheless cationic starch narrowed the number of CFU compared to those litter types without such an additive. The antimicrobial effect of Cationamyl 9865 was slightly higher than that of Cationamyl 9854. The sterile controls of all four litter types did not show microbial growth.

The growth behaviour of the filamentous fungi *Aspergillus brasiliensis* and *Trichoderma ssp.* was subsequently investigated (Exp 3). Both ONC showed similar optical densities. The sterile control was free of microbial growth. The growth control agar plate of *Aspergillus brasiliensis* showed a thin fungi layer after 48 h and total covering of the surface after six days of incubation. *Trichoderma ssp.* grew slower than *Aspergillus brasiliensis*. No colonies could be detected after 48 h. After six days a thin layer was visible on the plate. The sterile controls of the growth control and the litter resuspensions did not show microbial growth. After 96 h of incubation *Aspergillus brasiliensis* colonies were visible for all three litter resuspensions (cf. Exp 3 in figure 4.7). The 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9865 sample showed a fungal layer spreading across the whole agar surface. Resuspension agar plates of 5 wt% xanthan gum litter and 5 wt% xanthan gum, 1 wt% Cationamyl 9865 litter displayed only 103 and 81 colonies. This raised the question if the antimicrobial effect of the cationic starch was really present or if an error in the experimental procedure happened. The following experiment was aimed to clarify that. *Trichoderma ssp.* did not show fungal growth on any of the litter types. The growth conditions on the pellet surface were too harsh for this filamentous fungus.

The previous experiments showed that the bacterial and fungal test strains except *Aspergillus brasiliensis* were not able to grow on the pellet surface. Consequently, *Aspergillus brasiliensis* was solely used in this experiment. The ONC of *Aspergillus brasiliensis* had a similar optical density as in the experiments before. The sterile control of the ONC was free of microbial growth. The growth control agar plate was completely covered with a fungal layer after seven days of incubation. The sterile control of the growth control did not show fungal growth. The sterile controls of each litter type resuspension were clean too. Sterilization by dry heat was hence appropriate to prevent contamination with microorganisms that inherently lived on the pellet surface. Exp 4 in figure 4.7 shows that the fewest colonies grew at the 7 wt% xanthan gum sample, followed by the 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9865 litter. The two samples containing either 5 wt% xanthan gum or 5 wt% xanthan gum and 1 wt% Cationamyl 9865 displayed 110 and 150 colonies. The values for CFU per mL and per g were also twice as high for the latter two litter

types (cf. last section of table 4.6). This indicates that not only larger amounts of cationic starch had an antimicrobial effect, but also higher amounts of xanthan gum. This might result from improved water absorption, causing lower water activity.

4.4.2 Antimicrobial Effect of Cationic Starch in Liquid Media

To prove the antimicrobial action of cationic starch and to find the concentration limit of this effect, *Aspergillus brasiliensis* was grown in peptone water with different Cationamyl 9865 concentrations. Pictures of the blood agar plates used for evaluation can be seen in figure 4.8.

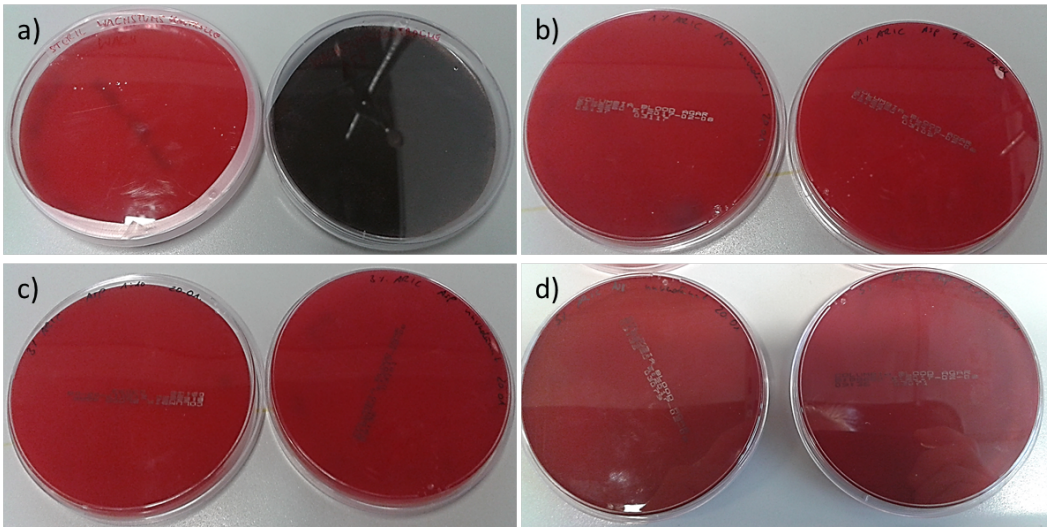

Figure 4.8: a) Sterile control (left) and growth control (right) of *Aspergillus brasiliensis*. Undiluted (left) and 10-fold diluted (right) sample of *Aspergillus brasiliensis* in medium containing b) 1 %(w/v) Cationamyl 9865 c) 3 %(w/v) Cationamyl 9865 and d) 5 %(w/v) Cationamyl 9865.

The negative control did not show microbial growth during the whole incubation time of eleven days. The agar plates hosting the positive control and a 10-fold dilution of the positive control were completely covered by an *Aspergillus brasiliensis* layer after 96 h. Therefore it was proven that no other microorganisms besides *Aspergillus brasiliensis* were present and that the fungus grew well in absence of cationic starch. The blood agar plates containing undiluted and 10-fold diluted aliquots of the 1 %(w/v), 3 %(w/v) and 5 %(w/v) Cationamyl 9865 samples did not show any defined colonies. However, small spots on the plates showing haemolysis indicated the presence of *Aspergillus brasiliensis* cells. After 96 h, 7 d and 11 d of incubation the growth process of the filamentous fungi was documented. The size of the haemolysis spots did not change throughout the whole incubation time. None of the plates showed a mycelium. Some cationic starch recrystallized on top of the agar plates. The 5 %(w/v) sample contained the highest amount of crystals. No

crystals were found on samples without cationic starch.

The antifungal effect of Cationamyl 9865 was proven without doubt. In liquid media 1 %(w/v) was enough to inhibit the growth of *Aspergillus brasiliensis* to a large extent. According to the previous experiments, 2.5 wt% of Cationamyl 9865 were necessary to reach an antimicrobial effect in solid samples.

Generally it can be said that the level of microbial activity was low throughout all cat litter types. On the pellets themselves neither bacterial nor fungal growth was observed. However, the growth control plates were fully covered by microbial layers proving that the pellets were inoculated with viable microorganisms. Undiluted aliquots of the pellet resuspension caused colony numbers in the range of 100. The referring CFU/g of cat litter never exceeded a range of 10^4 CFU/g. Janssen et al. reported 10^6 CFU/g to 10^7 CFU/g of dry pastureland soil [25]. Consequently, the microbial activity on the investigated cat litter samples was two to three orders of magnitude lower than that of soil. Uncontrollable microbial growth on the surface of the litter pellets was thus ruled out. Fecal bacteria like *E. coli* as well as the soil bacterium *Bacillus subtilis* and the mold fungus *Trichoderma ssp.* suffered most likely from the low water activity of the pellets. *Aspergillus brasiliensis* was less affected by this parameter. The antimicrobial effect was closely related to the amount of the clumping agent xanthan gum. The antifungal action of cat litter could be improved further by cationic starch. In this context Cationamyl 9865 showed better properties than Cationamyl 9854. However, cationic starch did not improve the clumping behaviour of the litter. This means that cationic starch can only be added to xanthan gum but cannot replace it. Liquid media containing 1 %(w/v) of Cationamyl 9865 inhibited the growth of *Aspergillus brasiliensis* almost completely. In all microbiological experiments the antifungal effect of Cationamyl 9865 was proven without doubt.

4.5 Sensory Evaluation

4.5.1 Descriptive Analysis of Cat Litter Samples

Descriptive analysis of different cat litter samples was performed to evaluate the sensory properties of the sawdust in combination with the additives and to get information on the odour differences perceived if synthetic cat urine was used or not. Eight members of the expert panel took part in this experiment. Figure 4.9 shows the terms used to describe the cat litter samples and their frequencies. Some of the terms have a frequency higher than 8. This is due to the fact that some panelists noted more than one word summarized in one group, e.g. “wood” and “sawdust”. A clear difference between samples prepared with deionized water and those prepared with synthetic cat urine could be found.

Wood scent was mainly noticed for samples prepared with deionized water and urine smell was almost exclusively perceived when synthetic cat urine was used. In case of 2.5 wt% xanthan gum, 2.5 % Cationamyl 9854 cat litter and *Commercial*

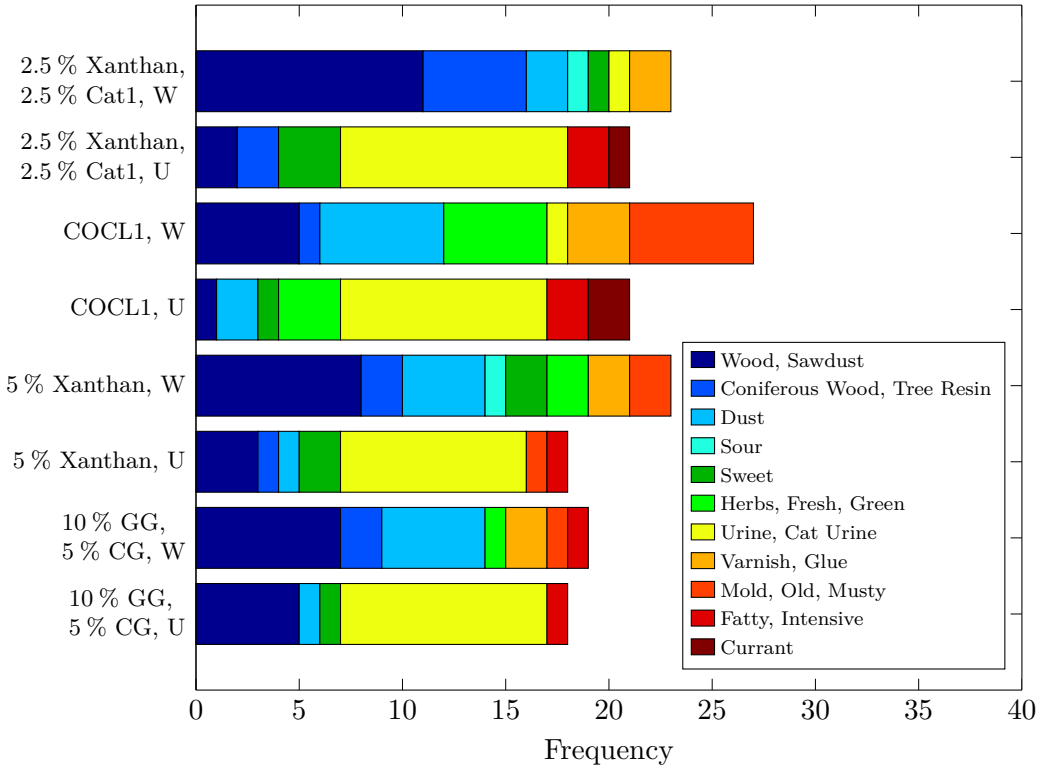

Figure 4.9: Stacked representation of the descriptive analysis of *Commercial Organic Cat Litter 1* and own cat litter samples prepared using synthetic cat urine (U) or deionized water (W). The additive concentration of the cat litter material is given in wt%. Cat1 = Cationamyl 9854, COCL1 = *Commercial Organic Cat Litter 1*, GG = Guar Gum, CG = Carob Gum.

Organic Cat Litter 1, urine smell was detected, even though the clumps were prepared with deionized water. In both cases the frequency of this term was 1. As retasting was allowed, it is possible that a sample containing synthetic cat urine was tried prior to the concerned samples. This caused that the urine scent was still in the panelist's nose when trying the sample with deionized water. However, it was undoubtedly proved that the formulation of the synthetic cat urine had an odour perceived as urine or cat urine.

"Dusty smell" was mainly recognized for samples prepared with deionized water. This term was found six times for *Commercial Organic Cat Litter 1*, four times for 5 wt% xanthan gum cat litter and five times for cat litter containing 10 wt% guar gum and 5 wt% carob gum.

"Fatty, intensive" odour was mentioned for three out of four samples prepared with synthetic cat urine. However, it was only mentioned once for litter clumps containing deionized water. These descriptions were assumed to be typical for the cat urine matrix.

Odour associated with varnish and glue was only perceived when samples were prepared with deionized water. In samples prepared with synthetic cat urine this smell was most likely covered by the more distinctive urine smell.

Some attributes appeared without recognizable accumulation throughout all samples, e.g. "coniferous wood, tree resin" or "sweet". Descriptions including words like "coniferous wood" or "tree resin" were noted zero to two times per sample. Only for the cat litter containing 2.5 wt% xanthan gum and 2.5 wt% Cationamyl 9854 the frequency of this term was 5, in case the clump was prepared with deionized water.

Some properties were rarely named. "Sour" was mentioned two times throughout all samples. "Herbal smell" or the attributes "fresh" and "green" were only mentioned for a few litter types. *Commercial Organic Cat Litter 1*, 5 wt% xanthan gum litter and 10 wt% guar gum, 5 wt% carob gum litter were described with these terms. Even though "herbs, fresh, green" was used five times for *Commercial Organic Cat Litter 1* nuggets with deionized water, also "mold, old, musty" was mentioned six times. *Commercial Organic Cat Litter 1* nuggets with synthetic cat urine got three hits for "herbs, fresh, green" aroma. A musty, old smell or the association with a mold-like aroma was typical for samples prepared with water. Three out of four samples emitted that smell. However, only one out of four samples prepared with synthetic urine evoked this perception.

The basic material and the additives had little malodours, as positive attributes like wood scent were predominately named.

4.5.2 Ranking Test with Friedman Analysis

Ranking tests with subsequent Friedman analysis were performed to evaluate the odour absorption ability of different additive types and concentrations. Therefore all cat litter clumps were prepared with synthetic urine. Figure 4.10 shows the statistical certainty of the significance of the ranking order.

The first sensory evaluation was performed in duplicates (R 1.1 and R 1.2). The intensity of the cat urine smell was perceived differently with a statistical certainty of 99.86 % in the first analysis and 99.98 % in the second analysis. The rank sums of the four samples are shown in figure 4.11. Tukey's honestly significant difference (HSD) in experiment R 1.1 was 14.82, and in R 1.2 it was 16.23. In experiment R 1.1 and R 1.2 the rank sums of *Commercial Organic Cat Litter 1* and the two cat litter samples containing xanthan gum differed by more than the HSD. The same was true for the 10 wt% guar gum, 5 wt% carob gum cat litter and the two xanthan gum samples. 5 wt% xanthan gum cat litter and 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9854 cat litter exhibited significantly lower intensities of cat urine smell. There was no statistically significant difference between 5 wt% xanthan gum cat litter and 2.5 wt% xanthan gum, 2.5 wt% Cationamyl 9854 cat litter as their rank sum difference was smaller than HSD. Also *Commercial Organic Cat Litter 1* and 10 wt% guar gum, 5 wt% carob gum cat litter did not show any statistically significant difference.

In the second ranking test (R 2) the probability value p was 0.1257. Therefore

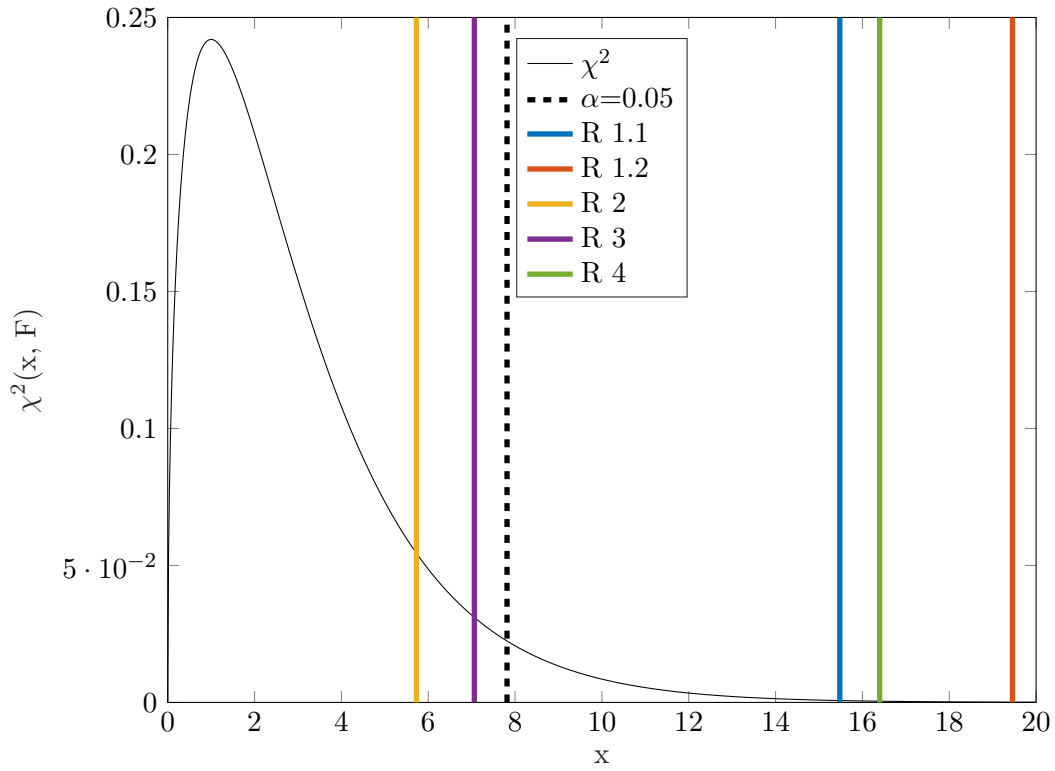

Figure 4.10: χ^2 distribution of all ranking tests. For a significance level of 95 % the alpha error α is 0.05. The statistical certainty s is given for each of the ranking tests.

the statistical certainty of a significant difference between the samples was only 87.43 %. Additionally, all rank sum differences were smaller than the calculated HSD of 15.54. Therefore the samples did not differ significantly in the intensity of the cat urine smell. It was concluded that neither activated carbon nor fumed silicon oxide particles improved the absorption of cat urine odour.

During the third ranking test (R 3) cat litter with different concentrations of xanthan gum were examined. With a value of 0.0700, p was larger than the α error. The statistical certainty of this ranking test was 93.00 %. This indicated that there was no significant difference in the intensity of the cat urine smell throughout the four samples. However, the rank sums of the litter with the highest additive concentration (7 wt% xanthan gum) and the lowest (3 wt% xanthan gum and 1 wt% Cationamyl 9865) differed by a value larger than the HSD of 16.90. The intensity of the cat urine smell was significantly lower in the 7 wt% xanthan gum cat litter than in the 3 wt% xanthan gum, 1 wt% Cationamyl 9865 cat litter. None of the other samples showed rank sum differences exceeding the HSD. In this experiment it was found that increasing concentrations of xanthan gum improved the odour absorption ability of cat litter.

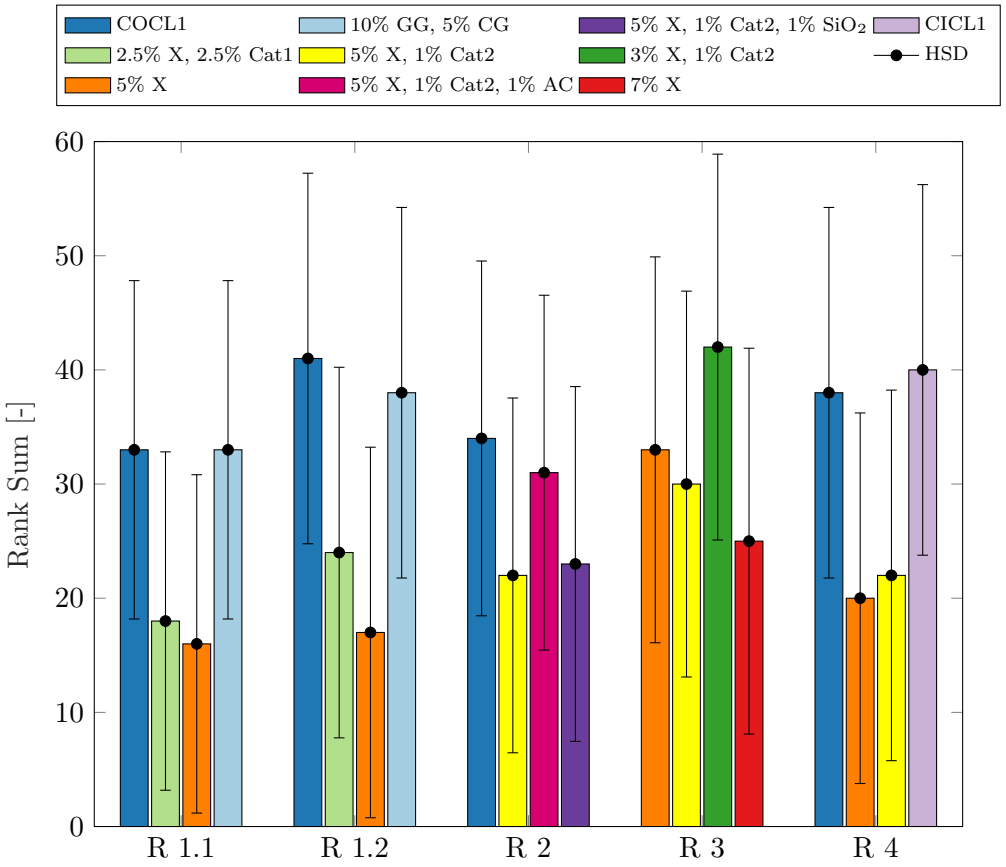

Figure 4.11: Rank sums and Tukey's honestly significant differences (HSD) of all ranking tests. The additive concentration is given in wt%. COCL1 = *Commercial Organic Cat Litter 1*, X = Xanthan, Cat1 = Cationamyl 9854, GG = Guar Gum, CB = Carob Gum, Cat2 = Cationamyl 9865, AC = Activated Carbon, SiO_2 = Fumed SiO_2 Particles, CICL1 = *Commercial Inorganic Cat Litter 1*.

Comparing *Commercial Organic Cat Litter 1* and *Commercial Inorganic Cat Litter 1* to 5 wt% xanthan gum cat litter and 5 wt% xanthan gum, 1 wt% Cationamyl 9865 cat litter resulted in a p value of 0.0009. The statistical certainty of a significant difference between the evaluated samples was 99.91 % (cf. R 4 in figure 4.10). Highly significant differences in the intensity of the cat urine smell were perceived for the four litter types. The rank sum differences of *Commercial Organic Cat Litter 1* and *Commercial Inorganic Cat Litter 1* were smaller than the HSD of 16.23. These two samples did not show a different odour absorption behaviour. 5 wt% xanthan gum cat litter and 5 wt% xanthan gum, 1 wt% Cationamyl 9865 cat litter did not differ from one another. Cat urine smell was significantly less intense in 5 wt% xanthan gum, 1 wt% Cationamyl 9865 cat litter than in *Commercial Inorganic Cat Litter 1*. Compared to *Commercial Organic Cat Litter 1* and *Commercial Inorganic Cat*

Litter 1 improved odour absorption abilities were found for cat litter containing 5 wt% of xanthan gum.

The descriptive analysis showed that litter clumps prepared with synthetic cat urine exhibit an odour associated with urine. This smell was almost not perceived for clumps prepared with deionized water. It could be concluded that the prepared synthetic cat urine authentically mimics the odour of aged cat urine. Sawdust and the additives showed little malodours. Dusty smell and a smell associated with varnish or glue were sometimes recognized. However, attributes having a positive connotation such as "wood, sawdust" and "coniferous wood, tree resin" were predominantly named. The first ranking test, performed in duplicates, showed that the intensity of cat urine smell was significantly lower in cat litter types containing xanthan gum than in *Commercial Organic Cat Litter 1* and cat litter containing guar gum and carob gum. Neither activated carbon nor fumed silicon oxide particles could improve the odour absorption ability of the produced cat litter types. However, increasing the concentration of xanthan gum decreased the intensity of the perceived cat urine smell. Compared to commercially available organic and inorganic cat litter brands odour absorption could be significantly improved in cat litter types containing xanthan gum as well as xanthan gum combined with cationic starch.

5 Summary and Outlook

In the present study a prototype of cat litter based on spruce sawdust was successfully developed. Additives fulfilling various consumer requirements on cat litter were identified. Solutions for stable clump formation, malodour absorption, high uptake capacity of watery liquids and antimicrobial action of the cat litter material were found.

Substances swelling only at elevated temperatures like starch were not suitable for the application as cat litter. As the body temperature of cats is only slightly higher than that of humans, the temperature of cat urine does not exceed 40 °C [28]. However, most starch types require at least temperatures of 60 °C for the initiation of the swelling process [9]. Guar gum, carob gum, xanthan gum and carboxymethylcellulose showed the ability to swell with cold water too. Xanthan gum was subsequently identified as the most suitable clumping agent. The formation of stable clumps was observed to be concentration dependent. Xanthan gum concentrations equal to and above 4 wt% resulted in stable clump formation. Below this concentration, handling of clumps with the sieve scoop was hardly possible, i.e. clumps broke apart when they were withdrawn from the litter box. In case of the reference litter types, without any additive, no defined clumps were formed.

The water uptake capacity of all xanthan containing cat litter types was similar to that of commercially available organic cat litter brands. Between 30 g and 40 g of dry litter material were necessary to absorb 50 mL of deionized water.

After the pelleting process one further comminution step was performed to obtain a particle size and shape suitable for organic cat litter. Different mechanical procedures like crushing, cutting and milling were considered. Cutting resulted in particle size distributions containing a large fraction of fine particles and only a small fraction of large particles being in the size range of 5 mm. Particle size distributions containing too many small particles can decrease the uptake velocity of cat urine and increase dust formation during cat litter handling. In this area further experiments considering other comminution techniques and particle separation methods need to be performed. However, the present results already provide a good basis for further fine tuning.

Few authors reported the composition of cat urine and the odorant volatile substances in it. To the author's knowledge, sensory evaluation of cat litter using odour imitating synthetic cat urine was not published by now. Cutting-edge results could be achieved in this part of the study. Descriptive analysis of the cat litter material, with and without cat urine, provided information about the inherent pellet odour as well as the characteristics of the prepared synthetic cat urine. The odour of pellets without cat urine was most frequently described as "wood", "sawdust", "coniferous

wood", "tree resin", "dust", "varnish" and "glue". Pellets with synthetic cat urine showed a distinctive urine and cat urine smell. In addition to that, attributes like "fatty", "intensive" and "currant" were used more often for this type of samples. Therefore the effectiveness of the odour imitating synthetic cat urine was proven without any doubt. Concerning the odour absorption ability of the litter material, it was found that higher xanthan gum concentrations enhance the odour absorption. This is most likely due to increased binding of liquids within the cat litter clumps, such that the odour active substances cannot contact the olfactory receptor cells and urine odour cannot be perceived. The produced cat litter samples were also compared to commercially available organic and inorganic cat litter types. In cat litter samples containing xanthan gum as well as xanthan gum and cationic starch type Cationamyl 9865 or type Cationamyl 9854, the intensity of the perceived cat urine smell was significantly lower than in commercially available products. Significant improvement of the odour absorption ability compared to well established cat litter brands was successfully demonstrated.

Plenty of patents claiming extraordinary good antimicrobial action of the respective cat litter types can be found. However, scientific reports dealing with qualitative and quantitative studies on microbial growth on cat litter have been rarely published yet. The reported investigations on the growing ability of different microbial species on the surface of the developed cat litter material and the quantification thereof delivered results with news value. Bacteria typically found in mammalian faeces like *E. coli* were not capable of surviving on the surface of wooden cat litter pellets. The same observation was made for *Bacillus subtilis*, a ubiquitously present soil bacterium that can be transferred to the cat litter box via the cat's fur or paws. Most likely the low water activity within the pellets was the reason of this effect. This hypothesis was also confirmed by the fact that other test microorganisms showed a decreased number of colonies at higher xanthan gum levels. This indicated that improved binding of liquid leading to lower water activity was directly responsible for the growth inhibition of microorganisms. The filamentous fungi *Aspergillus brasiliensis* was capable of surviving on the surface of the tested cat litter samples. However, it was never observed to form a visible mycel on the pellets themselves. Only after resuspension of the pellets in fresh media and plating aliquots of this suspension on blood agar plates showed that living *Aspergillus brasiliensis* spores were still present on the litter material. By either using cationic starch or by increasing the concentration of xanthan gum within the pellets it was possible to reduce the fungal growth level. It was further found that cationic starch of type Cationamyl 9865, which swelled with cold water, showed a higher antifungal effect than the cationic starch type Cationamyl 9854 that swelled only at elevated temperatures. Bacterial growth did not pose a serious problem for the developed organic cat litter. Filamentous fungi indeed needed to be considered. Their growth could be limited by the use of cationic starch or higher amounts of xanthan gum. Compared to pastureland soil (10^6 CFU/g to 10^7 CFU/g of dry soil) the microbial load of the produced cat litter material is two to four orders of magnitude lower as value between 10^3 CFU/g and 10^4 CFU/g were typically found.

The prototype of a competitive, environmentally friendly organic cat litter material was created in this work. Most relevant parameters including clumping ability, uptake capacity of watery liquids, odour absorption and antimicrobial effect could be solved by adding different types of additives. Excellent sensory properties with efficient malodour absorption could be achieved. Compared to competing organic cat litter brands the odour absorption ability was improved. In addition to that, microbial activity on the pellet surface was quantified and additives showing antimicrobial action were identified. The base material, spruce sawdust, as well as the additives xanthan gum and cationic starch are biodegradable. Xanthan gum is a product fermented from sugar bearing remainders of different industries, by bacteria of the genus *Xanthomonas*. This makes xanthan gum an additive which is largely independent of harvest, in contrast to guar gum or carob gum for which the success of harvest is critical. If allowed by the local authorities, a cat litter material as described in the current study would be compostable or could be discarded via the organic waste. In case this is not allowed and used organic cat litter has to be discarded via the residual waste, it can nevertheless be incinerated and used for energy production. At the same time, using organic cat litter instead of inorganic cat litter reduces the amount of waste stored on landfill sites. With the development of the described cat litter prototype a small contribution to improving the worldwide problems of environmental pollution and climate change could be made. In the future, cat litter based on spruce sawdust and xanthan gum will be a promising alternative to environmentally unfriendly inorganic cat litter types.

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List of Abbreviations

CFU	Colony Forming Unit
CMC	Carboxymethylcellulose
HSD	Tukey's Honestly Significant Difference
MFT	2-Methyl-3-furanthiol
MMB	3-Mercapto-3-methylbutan-1-ol
MMP	4-Methyl-4-mercaptopentan-2-one
MTE	2-(Methylthio)ethanol
OD_{600}	Optical Density at 600 nm
ODTV	Odour Detection Threshold Value, equals OPTV
ONC	Overnight Culture
OPTV	Odour Perception Threshold Value, equals ODTV
ORTV	Odour Recognition Threshold Value
OTV	Odour Threshold Value
PSD	Particle Size Distribution

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Appendix

Data of the Industrial Scale Pelleting Process

Table 5.1: Additives and their respective concentrations as well the water content of the sawdust, additive flow rate (AF), bulk density (BD) of the pellets, abrasion of the pellets, moisture of the pellets, pellet flow rate (PF) and frequency converter (FC) at 2300 kg/h press capacity are shown.

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Xanthan [wt%]	3	4	5	3	4	5
Sobocat [wt%]	-	-	-	1	1	1
Motor	RS248-82rpm					
Water [%]	12.28	12.28	12.28	12.28	12.28	12.28
AF [g/min]	1150	1533	1533	1917	1917	2300
BD [kg/m^3]	680	670	672	674	677	676
Abrasion [g]	1.02	1.16	1.02	1.07	0.83	0.84
Moisture [%]	8.9	8.5	8.8	8.7	8.3	8.6
PF [kg/h]	2300	2300	2300	2300	2300	2300
FC	1400	1850	2320	1850	2320	2780
Sampling	Big Bag					
Comment	All samples contain 0.5 wt% pelleting starch additionally. Cooling of the pellets is done outside for 10 min.					

AGRANA STÄRKE

PDB 9854 K de
Version 03, 31.10.12
Seite 1 von 1

CATIONAMYL 9854 K PRODUKTDATENBLATT
Papierstärke

ALLGEMEINE BESCHREIBUNG
- CATIONAMYL 9854 K ist ein Stärkeether auf Kartoffelbasis. Das Produkt ist heißwasserlöslich.

- Aussehen: weißes Pulver
- Geruch: leicht aminartig

ANALYSENWERTE
- Wassergehalt: 18,0 – 20,0 %
- Asche (800 °C): max. 2,0 % in TM
- pH-Wert (Aufkochung 2%i.TS, Raumtemperatur): 4,0 – 5,5
- Gebundener Stickstoff: 0,40 – 0,50 % in TM
- Viskosität: 2% in Trockensubstanz, Magnetrühreraufkochung (95 °C, 15 min. Haltezeit), Brookfield, Spindel 2/100 UpM,
  80 °C: 160 – 240 mPa.s
  50 °C: 200 – 280 mPa.s

LAGERUNG UND HALTBARKEIT
- Bei trockener Lagerung (max. 70 % relative Luftfeuchtigkeit) und vor Wärme geschützt: ca. 36 Monate

LIEFERFORM
- In mehrlagigen Papiersäcken zu 25 kg (1 Palette = 40 Säcke = 1000 kg)
- Big Bag zu 1000 kg
- In Silo-LKW

ZOLLTARIFNUMMER
- 3505 1050

EIGENSCHAFTEN UND ANWENDUNG
- CATIONAMYL 9854 K wurde speziell für die Papierindustrie entwickelt. Die Stärke wird hauptsächlich in der Masse (Pulper) bei der Papiererzeugung eingesetzt. Durch Verwendung von CATIONAMYL 9854 K wird eine bedeutende Verbesserung der Stärke-, Faser- und Füllstoffretention sowie eine Erhöhung der Festigkeit des Papiers erreicht.
- Andere bevorzugte Anwendungsgebiete sind die Karton- und Wellpapperohpapiererzeugung sowie die Abwasserreinigung in Papierfabriken.

AGRANA STÄRKE

CATIONAMYL 9865 PRODUKTDATENBLATT
Papierstärke

ALLGEMEINE BESCHREIBUNG
- CATIONAMYL 9865 ist ein kaltwasserlösliches Produkt auf Basis Kartoffelstärke.

• Aussehen:	weißgelbliches Pulver
• Geruch:	arteigen, rein

ANALYSENWERTE

• Wassergehalt:	4,0 – 8,0 %
• pH-Wert (5 % in S.):	6,0 – 9,0
• Gebundener Stickstoff (in TM):	min. 0,27 %
• Viskosität (5 % in TM Brookfield, Spindel 2/30 UpM):	200 – 600 mPas
• Siebanalyse:	max. 3, % > 1,0 mm

LAGERUNG UND HALTBARKEIT
- Bei trockener Lagerung (max. 70 % relative Luftfeuchtigkeit) und vor Wärme geschützt: ca. 36 Monate

LIEFERFORM
- Big Bag zu 500 kg

ZOLLTARIFNUMMER
- 3505 1050

EIGENSCHAFTEN UND ANWENDUNG
- CATIONAMYL 9865 wurde speziell für die Papierindustrie entwickelt. Die Stärke wird hauptsächlich in der Masse (Pulper) bei der Papiererzeugung eingesetzt. Durch Verwendung von CATIONAMYL 9865 wird eine bedeutende Verbesserung der Stärke-, Faser- und Füllstoffretention sowie eine Erhöhung der Festigkeit des Papiers erreicht.
- Andere bevorzugte Anwendungsgebiete sind die Karton- und Wellpapperohpapiererzeugung sowie die Abwasserreinigung in Papierfabriken.

Informationsdienst

SÜDSTÄRKE

Sobocat

Sobocat ist ein kationische, kaltwasserlösliche Kartoffelstärke für den Einsatz in der Masse bei Papier und Karton

Allgemeine Produktinformation:

Beschreibung:	helles, schwach gelbes, körniges Pulver, kaltwasserlöslicher, quartärer Stärkeether
Feuchtegehalt:	ca. 11 %
pH-Wert:	ca. 6
Stickstoffgehalt:	ca. 0,24 %

Eigenschaften:

Durch den Einsatz von **Sobocat** lassen sich die Faser-zu-Faser und die Faser-zu-Füllstoff-Bindekräfte entscheidend verbessern. Es wird eine Steigerung der Oberflächeneigenschaften und der inneren Festigkeit erreicht. Aufgrund der Kationenaktivität werden Mikroflocken gebildet. Die Entwässerung wird beschleunigt. Bei Erhöhung der Wasserführung, resultiert eine homogenere Blattbildung. Unter optimalen Voraussetzungen wird **Sobocat** vollständig durch ionische Adsorption an den Stoff gebunden. Deshalb wird im Siebwasser der CSB-Wert nicht beeinflußt. Die hohe Effektivität von **Sobocat** beruht auf einer statistisch gleichmäßigen Substitution an den einzelnen Stärkemolekülen.

Anwendung:

Zur Verbesserung und Unterstützung der Faser- und Füllstoffretention wird eine 1%ige oder noch weiter verdünnte Lösung kurz vor dem Stoffauflauf mit 0,4 - 0,8 % otro Stoff, eingesetzt. Zur ausgeprägten Verbesserung der Festigkeitseigenschaften wird, z. B. im Pulper, in der Mischbütte oder an anderer geeigneter Stelle mit 1 – 1,5 % oder mehr, otro Stoff, dosiert. Zur bevorzugten Anlagerung an Einzelkomponenten (Holzschliff, AP, Ausschuß) kann die **Sobocat** auch hier gezielt zugesetzt werden. Das Dosiersplitting kann die Wirkung potenzieren.

Herstellung der Lösungen:

Sobocat erspart das Kochen und kann mit Kaltwasser oder besser mit 30 – 50 °C warmem Wasser angesetzt werden. Nur eine vollständig gelöste kationische Stärke zeigt optimale Wirkung. Intensives Rühren und mäßig schnelles Einrühren verhindert die Bildung von Klumpen. Es empfiehlt sich, die Lösung durch ein Sieb (0,25 – 0,5 mm) zu filtrieren. Einfacher ist die Zugabe der ganzen wasserlöslichen Säcke im Pulper.

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www.suedstaerke.de – e-mail: info@suedstaerke.de

Produktspezifikation

Produkt	Xanthan 80 mesh
E-Nr.	415
Definition	Polysaccharid-Gummi, gewonnen durch Fermentation von Kohlehydraten mit einer Reinkultur von natürlich vorkommenden *Xanthomonas campestris*
Einsatzgebiet	Verdickungsmittel, Füllstoff, Geliermittel
Herkunft	China

Spezifikationswerte

Sensorische Beschreibung		
Aussehen		Weiß - cremefarben
Geruch und Geschmack		arteigen, einwandfrei
Chemische / Physikalische Daten		
pH - Wert (1 %ige Lösung)		6,0 – 8,0
Trocknungsverlust %(105 °C, 2,5 h)	max.	13,0
Asche gesamt % (in TM, 4 h 105 °C, 650 °C)	max.	13,0
Viskosität cps (1 %ig in KCl 1 %)		1.200 – 1.600
Korngröße mesh	ca.	80
Gehalt % (in Trockensubstanz)		91,0 – 108,0
Brenztraubensäure %	min.	1,5
Stickstoff %	max.	1,5
V1:V2		1.020 – 1.450
Löslichkeit		in Wasser löslich in Ethanol unlöslich
Ethanol und Propan-2-ol ppm		500, einzeln oder zusammen
Schwermetalle ppm	max.	20,0
Arsen ppm	max.	3,0
Blei ppm	max.	2,0
Mikrobiologische Daten		
Gesamtkeimzahl p/g	max.	5.000
Hefen und Schimmelpilze p/g	max.	200
E.coli /5 g		negativ
Salmonellen /10 g		negativ
Xanthomonas Campestris		keine lebensfähigen Zellen in 1 g

Verpackung	Kartons à 25 kg
Haltbarkeit	2 Jahre nach Herstellungsdatum
Lagerung	Kühl und trocken lagern, Gebinde fest verschlossen, bei 15-28°C, max. 65% RH

Das Material entspricht den Anforderungen des Food Chemical Codex (FCC), der EU Verordnung 231/2012 mit Spezifikationen für die in den Anhängen II und III der Verordnung (EG) Nr. 1333/2008 aufgeführten Lebensmittelzusatzstoffe und der EU Richtlinie 1831/2003/EG über Zusatzstoffe in der Tierernährung.

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zuletzt geändert am: 28.01.2014
Erstellt durch: QS
Seite **1** von **3**

Produktspezifikation

1. Inhaltsstoffe mit allergenem Potential gemäß der Verordnung (EU) Nr. 1169/2011

Angabe des allergieauslösenden Bestandteils unter genauer Nennung der entsprechenden Zutat

Xanthan

	enthalten u./o. zugesetzt			Im Produktionsstandort eingesetzt, bzw. transport- oder lagerrelevant			Kreuzkontamination möglich?	
	nein	ja	wenn ja, genaue Bezeichnung	nein	ja	wenn ja, genaue Bezeichnung	nein	ja
1.1 Glutenhaltiges Getreide (d.h. Weizen, Roggen, Gerste, Hafer, Dinkel, Kamut oder Hybridstämme davon) sowie daraus hergestellte Erzeugnisse	☒	☐		☒	☐		☒	☐
1.2 Krebstiere und Krebstiererzeugnisse	☒	☐		☒	☐		☒	☐
1.3 Eier und Eierzeugnisse	☒	☐		☒	☐		☒	☐
1.4 Fisch und Fischerzeugnisse	☒	☐		☒	☐		☒	☐
1.5 Erdnüsse und Erdnusserzeugnisse	☒	☐		☒	☐		☒	☐
1.6 Soja und Sojaerzeugnisse	☒	☐		☒	☐		☒	☐
1.7 Milch und Milchererzeugnisse (einschließlich Lactose)	☒	☐		☒	☐		☒	☐
1.8 Schalenfrüchte (d.h. Mandel, Haselnuss, Walnuss, Kaschunuss, Pecannuss, Paranuss, Pistazie, Macadamianuss und Queenslandnuss) sowie daraus hergestellte Erzeugnisse	☒	☐		☒	☐		☒	☐
1.9 Sellerie und Sellerieerzeugnisse	☒	☐		☒	☐		☒	☐
1.10 Senf und Senferzeugnisse	☒	☐		☒	☐		☒	☐
1.11 Sesamsamen und Sesamsamenerzeugnisse	☒	☐		☒	☐		☒	☐
1.12 Schwefeldioxid und Sulfite in einer Konzentration von mehr als 10 mg/kg oder 10 mg/l, als SO_2 angegeben	☒	☐		☒	☐		☒	☐
1.13 Lupinen und daraus gewonnene Erzeugnisse	☒	☐		☒	☐		☒	☐
1.14 Weichtiere und daraus gewonnene Erzeugnisse	☒	☐		☒	☐		☒	☐

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Erstellt durch: QS
Seite **2** von **3**

Produktspezifikation

Nährwerte	Pro 100 g
Brennwert KJ	759
Brennwert Kcal	188
Kohlenhydrate g	0
davon Zucker g	0
Ballaststoffe g	80
Fett (total) g	0
Cholesterin g	0
Protein g	7
Natrium mg	50
Kalium mg	42
Calcium mg	800

*Alle Angaben unterliegen den bei Naturprodukten üblichen Schwankungen.

NON-GMO Statement

Hiermit bestätigen wir, dass o.g. Produkt nicht aus genetisch veränderten Organismen (GVO) besteht, keine enthält oder daraus hergestellt wird, und dass im gesamten Produktionsprozess, keine Rohstoffe verwendet werden, die als GVO zu kennzeichnen sind oder der Rückverfolgbarkeit unterliegen.

Weiterhin bestätigen wir,

dass die für Lebens- und Futtermittel geltenden Vorschriften für Rückverfolgbarkeit (Verordnung (EG) Nr. 1830/2003) und die Verordnung (EG) Nr. 1829/2003 des Europäischen Parlaments und des Rates vom 22. September 2003 über genetisch veränderte Lebens- und Futtermittel, von uns stets eingehalten werden.

Materialien / Gegenstände

Hiermit bestätigen wir, dass o.g. Produkt, der Verordnung (EG) Nr. 1935/2004 vom 27. Oktober 2004 über Materialien und Gegenstände, die dazu bestimmt sind, mit Lebensmitteln in Berührung zu kommen und zur Aufhebung der Richtlinien 80/590/EWG und 89/109/EWG sowie der Verordnung (EU) Nr. 10/2011 vom 14. Januar 2011 über Materialen und Gegenstände aus Kunststoff, die dazu bestimmt sind, mit Lebensmitteln in Berührung zu kommen, entspricht.

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Protokoll

bioenergy2020+

Mitarbeiter:	Pointner	weitere Notizen (Grund der Pelletierung):
Datum:	13.10. und 12.12./13.12.2016	Fichtenpellets mit verschiedenen
Projekt:	Hasslacher Direktauftrag	Additiven

Rohstoff / Additive (wie angeliefert)	Wassergehalt [%]	Schüttdichte [kg/m³]	Korngröße [mm]	Notizen
Fichtensägespäne	10,87	185,8	4,0	Hasslacher Holzindustrie **Rohstoff verunreinigt mit Sieb und Schleifstaub!**
Xanthan Gum				Fa. Buxtrade GbR
Johannisbrotkernmehl				
kationiesierte Stärke				
Aktivkohle, Pulver	0,0		< 1	Fa. Roth, Art.Nr. X865.2
Silikagel, Pulver				VWR
Guarkernmehl				

Notizen zum Rohstoff:
Sägespäne aus Sägewerk Hasslacher in BigBags angeliefert
Wassergehalt Pellets vor Kühlung (heißer Zustand)!

Versuch 01: 13.10.2016 — Archiev Nr.: P16-007.01

1. Matrize:
Lochdurchmesser: 6 [mm]
Pressweg: 27 [mm]
Pressverhältnis: 1:4,5 [-]

2. Aufgabematerial:
Fichtensägespäne 100 [%]
[%]
[%]
Wassergehalt: 14,00 [%]
Heizwert ($H_{u,wf}$): 18,00 [MJ/kg TS]
Körnung: 4 [mm]
Schüttdichte ($d_{i,roh}$): 188,0 [kg/m³]

keine zerkleinerung notwendig

3. Pelletierverlauf:
Pelletierung möglich: Ja [-]
Temperatur: 96,0 [°C]
Dosiereinrichtung: 600 [U/min]
Kollerradeinstellung: 3,8 [Stufe]
Durchsatz: 245 [g/min]
Leistungsaufnahme: 1,50 [kW]

OPTIMAL, max. Drehzahl Dosiereinrichtung bei mittlerer Leistungsaufnahme, ideale Matrizenbelastung bei gutem Einzug, leichtes Knattern, mit Deckel und Stutzen, Dampfaustritt bei Auswurf, Anzugsmoment Koller: 80 Nm, konstante Prozessbedingungen

4. Pelletsqualität:
Wassergehalt: 9,94 [%]
Feinanteil: 0,10 [%]
Schüttdichte: 583,1 [kg/m³]
Abrieb: -- [%]

feinste Qualität, glänzend, geschlossen, lange, stabil ohne Feinanteil

5. Rückstellprobe: vorhanden? Nein

6. Anmerkungen:
Matrize neu, Einzug tief oben
Anzugsmoment: 80Nm

Versuch 02: 13.10.2016 — Archiev Nr.: P16-007.02

1. Matrize:
Lochdurchmesser: 6 [mm]
Pressweg: 27 [mm]
Pressverhältnis: 1:4,5 [-]

2. Aufgabematerial:
Fichtensägespäne 98 [%]

6. Anmerkungen:
Matrize neu, Einzug tief oben
Anzugsmoment: 80Nm

Protokoll

bioenergy2020+

	Xanthan Gum	2	[%]	
			[%]	
	Wassergehalt:	13,30	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	188,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	geht fein, jedoch ohne Kappe - etwas zu viel Wasser, Additiv hat gelierende Wirkung, Prozessparameter und Einstellungen unverändert
	Temperatur:	96,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	3,8	[Stufe]	
	Durchsatz:	255	[g/min]	
	Leistungsaufnahme:	1,70	[kW]	
4. Pelletsqualität:	Wassergehalt:	10,39	[%]	noch besser als zuvor!
	Feinanteil:	0,10	[%]	
	Schüttdichte:	610,7	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 03: 13.10.2016 **Archiev Nr.:** **P16-007.03**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	Retsch Schneidmühle SM-100
	Xanthan	5	[%]	Temperatur:
			[%]	Druchsatz:
	Wassergehalt:	13,16	[%]	spez. Energiebedarf:
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	188,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	ohne Kappe, optimal
	Temperatur:	98,0	[°C]	
	Dosiereinrichtung:	650	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	265	[g/min]	
	Leistungsaufnahme:	1,90	[kW]	
4. Pelletsqualität:	Wassergehalt:	10,17	[%]	optimal, extrem lange, Oberfläche etwas "klebrig"+F24
	Feinanteil:	0,10	[%]	
	Schüttdichte:	609,3	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 04: 13.10.2016 **Archiev Nr.:** **P16-007.04**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	kein Aufmahlen notwendig
	Xanthan	4,5	[%]	

Protokoll

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	Johannesbrotkernmeł	0,5	[%]	
	Wassergehalt:	13,10	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	knattern etwas lauter, ohne Kappe, geht nicht besser
	Temperatur:	97,0	[°C]	
	Dosiereinrichtung:	650	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	280	[g/min]	
	Leistungsaufnahme:	1,90	[kW]	
4. Pelletsqualität:	Wassergehalt:	9,50	[%]	Pippifein, jedoch Oberfläche etwas "klebrig"
	Feinanteil:	0,10	[%]	
	Schüttdichte:	603,8	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 05: 13.10.2016 **Archiev Nr.:** **P16-007.05**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	kein Bedarf
	Xanthan	2,5	[%]	
	Kartoffelstärke	2,5	[%]	
	Wassergehalt:	12,90	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	unverändert - gleich Versuch 3 und 4
	Temperatur:	97,0	[°C]	
	Dosiereinrichtung:	650	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	290	[g/min]	
	Leistungsaufnahme:	1,70	[kW]	
4. Pelletsqualität:	Wassergehalt:	10,18	[%]	top, nicht mehr so klebrig
	Feinanteil:	0,10	[%]	
	Schüttdichte:	606,2	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 06: 13.10.2016 **Archiev Nr.:** **P16-007.06**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	kein Bedarf
	Xanthan	2,5	[%]	
	Stärke 1: Cat 9865	2,5	[%]	

Protokoll

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	Wassergehalt:	12,65	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	unverändert, kein Knattern
	Temperatur:	100,0	[°C]	
	Dosiereinrichtung:	650	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	275	[g/min]	
	Leistungsaufnahme:	1,90	[kW]	
4. Pelletsqualität:	Wassergehalt:	9,44	[%]	optimal
	Feinanteil:	0,10	[%]	
	Schüttdichte:	610,0	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 07: 13.10.2016 **Archiev Nr.:** **P16-007.07**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	kein Bedarf
	Xanthan	2,5	[%]	
	Stärke 2: Cat 9854	2,5	[%]	
	Wassergehalt:	13,10	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	unverändert, jedoch deutliches Knattern, leichter Temperatur- und Leistungsrückgang
	Temperatur:	97,0	[°C]	
	Dosiereinrichtung:	650	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	265	[g/min]	
	Leistungsaufnahme:	1,70	[kW]	
4. Pelletsqualität:	Wassergehalt:	10,19	[%]	optimal
	Feinanteil:	0,10	[%]	
	Schüttdichte:	594,5	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 08: 13.10.2016 **Archiev Nr.:** **P16-007.08**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	85	[%]	kein Bedarf
	Guakernmehl	10	[%]	
	Johannesbrotkernmeh	5	[%]	
	Wassergehalt:	13,00	[%]	

Protokoll

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	Heizwert ($H_{u,wf}$):		18,00	[MJ/kg TS]
	Körnung:		4	[mm]
	Schüttdichte ($d_{i,roh}$):		190,0	[kg/m³]
3. Pelletierverlauf:	Pelletierung möglich:	Ja		[-]
	Temperatur:		94,0	[°C]
	Dosiereinrichtung:		500	[U/min]
	Kollerradeinstellung:		3,5	[Stufe]
	Durchsatz:		205	[g/min]
	Leistungsaufnahme:		1,20	[kW]
4. Pelletsqualität:	Wassergehalt:		9,20	[%]
	Feinanteil:		0,50	[%]
	Schüttdichte:		573,1	[kg/m³]
	Abrieb:		--	[%]
5. Rückstellprobe:	vorhanden?	Nein		

zu geringer Presswiderstand aufgr. hoher Additivmenge, geringere Matrizentemperatur, gleichmäßige Belastung, kein Knattern, sehr leichter Einzug, ohne Kappe, --> für bessere Qualität höheres Pressverhältnis

glänzend, raue, rissige Oberfläche, wenig stabil, hellere Farbe, etwas Feinanteil

Versuch 09: 12.12.2016 **Archiev Nr.:** **P16-007.09**

1. Matrize:	Lochdurchmesser:		6	[mm]
	Pressweg:		27	[mm]
	Pressverhältnis:		1:4,5	[-]
2. Aufgabematerial:	Fichtensägespäen		96	[%]
	Xanthan		3	[%]
	Stärke 1: Cat 9865		1	[%]
	Wassergehalt:		12,63	[%]
	Heizwert ($H_{u,wf}$):		18,00	[MJ/kg TS]
	Körnung:		4	[mm]
	Schüttdichte ($d_{i,roh}$):		190,0	[kg/m³]
3. Pelletierverlauf:	Pelletierung möglich:	Ja		[-]
	Temperatur:		100,0	[°C]
	Dosiereinrichtung:		600	[U/min]
	Kollerradeinstellung:		4,0	[Stufe]
	Durchsatz:		290	[g/min]
	Leistungsaufnahme:		2,10	[kW]
4. Pelletsqualität:	Wassergehalt:		9,38	[%]
	Feinanteil:		0,01	[%]
	Schüttdichte:		619,5	[kg/m³]
	Abrieb:		--	[%]
5. Rückstellprobe:	vorhanden?	Nein		

6. Anmerkungen:
Matrize neu, Einzug tief oben
Anzugsmoment: 80Nm

Rohstoff nicht gemahlen - Pelletiert wie angeliefert; Rohstoff abgemischt und ca. 1h Reifezeit im Mörteltrog

OPTIMAL, max. Drehzahl Dosiereinrichtung bei mittlerer Leistungsaufnahme, ideale Matrizenbelastung bei gutem Einzug, kaum bis leichtes Knattern, ohne Deckel, mit Stutzen, Dampfaustritt bei Auswurf, Anzugsmoment Koller: 80 Nm, konstante

feinste Qualität, glänzend, geschlossen, lange, stabil ohne Feinanteil, Oberfläche etwas klebrig; Wassergehalt im noch heißen Zustand

Protokoll

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Versuch 10: 12.12.2016 **Archiev Nr.:** **P16-007.10**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	94	[%]	
	Xanthan	4	[%]	
	Stärke 1: Cat 9865	1	[%]	
	Wassergehalt:	12,35	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	Einstellungen und Parameter unverändert!
	Temperatur:	97,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	290	[g/min]	
	Leistungsaufnahme:	2,00	[kW]	
4. Pelletsqualität:	Wassergehalt:	8,05	[%]	Optimal - wie vorher Versuch 10
	Feinanteil:	0,01	[%]	
	Schüttdichte:	613,4	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 11: 12.12.2016 **Archiev Nr.:** **P16-007.11**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	95	[%]	Rohstoff wie angeliefert, in Freifallmischer konditioniert und 1h im Mörtelkasten zur Reife
	Xanthan	5	[%]	
	Stärke 1: Cat 9865	1	[%]	
	Wassergehalt:	12,60	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	187,7	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	alles unverändert, sehr gut pelletierbar
	Temperatur:	98,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	320	[g/min]	
	Leistungsaufnahme:	2,10	[kW]	
4. Pelletsqualität:	Wassergehalt:	8,35	[%]	optimalst, Pellets extrem hart
	Feinanteil:	0,01	[%]	
	Schüttdichte:	614,1	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Protokoll

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Versuch 12: 12.12.2016 **Archiev Nr.:** **P16-007.12**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	93	[%]	Rohstoff wie angeliefert, in Freifallmischer konditioniert und 1h im Mörtelkasten zur Reife
	Xanthan	7	[%]	
			[%]	
	Wassergehalt:	12,53	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	keine Veränderung
	Temperatur:	98,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	285	[g/min]	
	Leistungsaufnahme:	2,00	[kW]	
4. Pelletsqualität:	Wassergehalt:	9,73	[%]	unverändert
	Feinanteil:	0,01	[%]	
	Schüttdichte:	613,8	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 13: 12.12.2016 **Archiev Nr.:** **P16-007.13**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	93	[%]	Rohstoff wie angeliefert, in Freifallmischer konditioniert und 1h im Mörtelkasten zur Reife
	Xanthan	5	[%]	
	Aktivkohle	1	[%]	
	Stärke 1: Cat 9865	1	[%]	
	Wassergehalt:	12,82	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	Anzugsmoment: 80 Nm geht fein, etwas stärker schwergängiger als vorherige, mit Kappe, Temperatur Matrize um 10°C höher, guter Einzug und Matrizenbelastung, ruhiger Kollergang
	Temperatur:	105,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,5	[Stufe]	
	Durchsatz:	265	[g/min]	
	Leistungsaufnahme:	2,40	[kW]	
4. Pelletsqualität:	Wassergehalt:	7,08	[%]	sehr gute Qualität, jedoch durch Aktivkohle grau bis schwarz
	Feinanteil:	0,01	[%]	
	Schüttdichte:	587,9	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Protokoll

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Versuch 14: 12.12.2016 **Archiev Nr.:** **P16-007.14**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	93	[%]	Rohstoff wie angeliefert, in Freifallmischer konditioniert und 1h im Mörtelkasten zur Reife
	Xanthan	5	[%]	
	Fumed Silica	1	[%]	
	Stärke 1: Cat 9865	1	[%]	
	Wassergehalt:	12,60	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	optimal, Temperatur leicht rückläufig
	Temperatur:	105,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	285	[g/min]	
	Leistungsaufnahme:	2,10	[kW]	
4. Pelletsqualität:	Wassergehalt:	7,99	[%]	optimal
	Feinanteil:	0,01	[%]	
	Schüttdichte:	620,7	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

Versuch 15: **Referenzprobe 13.12.2016** **Archiev Nr.:** **P16-007.15**

1. Matrize:	Lochdurchmesser:	6	[mm]	**6. Anmerkungen:**
	Pressweg:	27	[mm]	Matrize neu, Einzug tief oben
	Pressverhältnis:	1:4,5	[-]	Anzugsmoment: 80Nm
2. Aufgabematerial:	Fichtensägespäne	100	[%]	Rohstoff wie angeliefert, in Freifallmischer konditioniert und 1h im Mörtelkasten zur Reife
			[%]	
			[%]	
	Wassergehalt:	13,15	[%]	
	Heizwert ($H_{u,wf}$):	18,00	[MJ/kg TS]	
	Körnung:	4	[mm]	
	Schüttdichte ($d_{i,roh}$):	190,0	[kg/m³]	
3. Pelletierverlauf:	Pelletierung möglich: Ja		[-]	Prozessparameter unverändert, kein Unterschied zu vorherigen Proben, jedoch mit Deckel
	Temperatur:	100,0	[°C]	
	Dosiereinrichtung:	600	[U/min]	
	Kollerradeinstellung:	4,0	[Stufe]	
	Durchsatz:	280	[g/min]	
	Leistungsaufnahme:	2,00	[kW]	
4. Pelletsqualität:	Wassergehalt:	9,40	[%]	
	Feinanteil:	0,01	[%]	
	Schüttdichte:	620,7	[kg/m³]	
	Abrieb:	--	[%]	
5. Rückstellprobe:	vorhanden? Nein			

VWR BDH PROLABO CHEMICALS

CERTIFICATE OF ANALYSIS

PRODUCT DATA

Description	***Columbia Blood Agar***
Product code	100253ZF
Batch	88650
Presentation	20 Prepared Plates/90 mm
Expiry	08/02/2017
Manufacturing	25/11/2016

COMPOSITION in g / L

Composition (g/l);

Casein pancreatic digest	10.0
Meat peptic digest	5.00
Heart Pancreatic digest	3.00
Yeast Extract	5.00
Sodium chloride	5.00
Starch	1.00
Agar	15.0
Defibrinated Sheep blood	50.0 ml

PHYSICAL-CHEMICAL TEST

Color	Red
pH	7,3 (7,2 ± 0,2)
Weight	22 (22 ± 2 g)

Result: **Satisfactory**

STERILITY TEST

Incubation 48 hours at 30-35ºC and 48 hours at 20-25ºC: NO GROWTH

Result: **Satisfactory**

Check at 7 days after incubation in same conditions

FERTILITY TEST

Inoculate: Practical range 100±20 CFU; Min. 50 CFU (Productivity)/ 10^4-10^6 (Selectivity).

5% CO2 atmosphere. Incubation at 37 ºC during 24-48 h.

Microorganism	Specification	CFU	Recovery
Staphylococcus aureus ATCC® 25923	Good Beta-haemolysus- Clear halo	112	100%
Enterococcus faecalis ATCC® 19433	Good Gamma haemolysis- Without halo	43	83%
Streptococcus pneumoniae ATCC® 49619	Good Alpha haemolysis- Greenish halo	55	90%
Streptococcus pyogenes ATCC® 19615	Good Beta-haemolysus- Clear halo	91	95%
Escherichia coli ATCC® 25922	Good Beta-haemolysus- Clear halo	66	89%
Streptococcus agalactiae ATCC® 12386	Good Beta-haemolysus- Clear halo	77	90%

*Microbiological quality criteria according to European Pharmacopoeia. Maximum acceptable count = 200 CFU

Result: **Satisfactory**

Storage conditions: 2-14ºC
Avoid direct contact with surfaces that can freeze product.

This certificate is an electronic copy of the certificate available in our laboratory and does not require signature.

VWR International bvba/sprl
Haasrode Research park Zone 3
Geldenaaksebaan 464
b-3001 Leuven Tel +32 (0) 16 385 011

Quality release date: 28/11/2016

VWR BDH PROLABO CHEMICALS

CERTIFICATE OF ANALYSIS

PRODUCT DATA

Description	***Buffered Peptone Water - 9 ml***
Product code	610173ZA
Batch	86847
Presentation	20 Tubes /Tube 16 x 113 mm
Expiry	25/08/2017
Manufacturing	30/08/2016

COMPOSITION in g / L

Composition (g/l):	
Peptone	10.0
Sodium chloride	5.00
Disodium phosphate12 H_2O	9.00
Potassium phosphate	1.50

PHYSICAL-CHEMICAL TEST

Color	yellow
pH	7,1 (7 ± 0,2)
Weight	9 (9 ± 0,05 g)

Result: **Satisfactory**

STERILITY TEST

Incubation 48 hours at 30-35ºC and 48 hours at 20-25ºC: NO GROWTH

Result: **Satisfactory**

Check at 7 days after incubation in same conditions

FERTILITY TEST

Prepare tubes / Inoculate 10^3- 10^4 (Productividad)/ subculture to T0, 45 minutes, 1h at 20-25ºC;
Microbiological control according to ISO 11133:2014

Aerobiosis. Incubation at 35 ± 2ºC. Reading at 24 hours.

Microorganism	Specification	Inoculated CFU	Results
Candida albicans ATCC® 10231	Good. Recovery ±30% T0 (original enumeration)	95	Conforms
Escherichia coli ATCC® 8739	Good. Recovery ±30% T0 (original enumeration)	75	Conforms
Salmonella typhimurium ATCC® 14028	Good. Recovery ±30% T0 (original enumeration)	95	Conforms
Staphylococcus aureus ATCC® 25923	Good. Recovery ±30% T0 (original enumeration)	72	Conforms
Pseudomonas aeruginosa ATCC® 9027	Good. Recovery ±30% T0 (original enumeration)	90	Conforms
Listeria monocytogenes ATCC® 13932	Good. Recovery ±30% T0 (original enumeration)	105	Conforms

Result: **Satisfactory**

This certificate is an electronic copy of the certificate available in our laboratory and does not require signature.

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Quality release date: 05/09/2016

Sensorische Beurteilung von Katzenstreu

Name	
Prüfnummer	

Auf dem Prüfplatz stehen 4 mal 2 Proben Katzenstreu. Je 2 Proben gehören zusammen. In den mit W gekennzeichneten Proben wurden die Klumpen durch Zusatz von Wasser erzeugt – bei den zugehörigen Proben ‚ohne W' unter Zusatz von synthetischem Katzenurin. Vergleiche bitte die zusammengehörigen Proben und beschreibe den Geruch!

	Geruch	Geruch
Serie 1	**W 318**	**318**
Serie 2	**W 256**	**256**
Serie 3	**W 498**	**498**
Serie 4	**W 503**	**503**

Bitte nach dem Beenden der Prüfung alle Becher mit einem Deckel verschließen!

Rangordnungsprüfung

Name	
Prüfnummer	

Prüfanleitung

Auf dem Platz stehen 4 Proben Katzenstreu. Alle Proben wurden mit synthetischem Katzenurin hergestellt. Versuche bitte, die Proben hinsichtlich der Intensität des Katzenurin-Geruchs zu reihen. Rückkosten ist erlaubt!

am wenigsten intensiv	→	→	am intensivsten

Bitte nach dem Beenden der Prüfung alle Becher mit einem Deckel verschließen!