Influence of Extrusion Parameters and Recipe Compounds on Flavor Formation and its Detection and Quantification

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"So eine Arbeit ist eigentlich nie fertig,

man muss sie für fertig erklären,

wenn man nach Zeit und Umständen das Möglichste getan hat."

(J.W. Goethe; „Italienische Reise", 1787)

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Abbreviations

APCI	atmospheric pressure chemical ionization
AS	amino acid
CI	chemical ionization
DHS	dynamic headspace sampling
EI	electron ionization
ESI	electrospray ionization
FD	field desorption
FID	flame ionization detector
FPD	flame photometric detector
GC	gas chromatograph / gas chromatography
GC-MS	gas chromatography-mass spectrometry
GLC	gals-liquid chromatography
HPLC	high performance liquid chromatography
HTST	high-temperature short-time
L*	lightness
L	relative lightness (100-L*)
LC	liquid chromatography
LLE	liquid-liquid extraction
m/z	dimensionless mass to charge ratio
MALDI	matrix assisted laser desorption/ionization
MP/CS	milk protein/glucose syrup
MP/G	milk protein/glucose
MS	mass spectromter / mass spectrometry
MS/MS	tandem mass spectrometry
MSD	mass selective detector
N	Nitrogen
NIR	near infrared reflectance
NIT	near infrared transmittance
NPLC	normal phase liqid chromatography
PID	photo ionization detector
ppm	parts per million
RPLC	reversed phase liquid chromatography
rt	retention time
RTE	ready to eat
SAFE	solvent assisted flavor evaporation
SDE	simultaneous distillation-extraction
SIDA	stable isotope dillution analysis
SIM	selctive ion monitoring
SME	specific mechanical energy
TCD	thermal conductivity detector
TMP	trimethylpyrazine
TOF	time of flight
VHS	vacuum headspace
WP/G	whey protein/glucose

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Summary

Objective of the dissertation was to examine the influence of specific Maillard reaction precursors and temperature on the formation of flavor compounds and color development in extrusion cooked food products. For this, functional relationships between process, system, and product parameters were analyzed.

Dry mixtures of wheat starch and protein were processed in a 44 mm diameter, co-rotating twin screw extruder by the addition of sugar and single amino acids dissolved in water. For each mixture of protein and sugar, a 3^3 factorial experiment was designed on the following operating conditions:

i) barrel temperature 140°C, 160°C, and 180°C

ii) protein concentration of the mixture 1.3%, 2.0%, and 2.6%

iii) sugar concentration of the mixture 2%, 4%, and 6%

Screw speed, moisture, and feed rate were kept constant to narrow the influencing variables and to keep the residence time unchanged.

Protein and sugar concentrations and barrel temperature showed different influences on the system parameters die temperature, die pressure, and specific mechanical energy input (SME). SME was found to be the only system parameter showing a significant influence on flavor development. An increase resulted in elevated concentrations of pyrazines and furanmethanol, representing important flavor compounds. Within this study, no statistical significance of product pressure and product temperature could be found regarding their influence on the formation of the examined flavor compounds.

Evaluations of the influence of protein, sugar, and temperature as well as separate examinations of the influence of single amino acids are based on GC/MS quantification results obtained by stable isotope dilution analysis (SIDA) or the use of trimethypyrazine (TMP).

The results showed distinct differences in how protein, sugar, and temperature influence different groups of flavor compounds. It was found that the most important flavor compounds for the desired cereal like aroma among the evaluated compounds, i.e. pyrazines, furanmethanol, and maltol, showed similarities in how they were generally affected. They were significantly positive influenced by increasing barrel temperatures and protein contents. Increasing sugar contents led to decreasing concentrations of 2,5-dimethylpyrazine, furanmethanol, and maltol whereas the formation of methylpyrazine was favored up to 4% sugar followed by a distinct drop at higher concentrations. The kind of protein and sugar used was found to be important. The formation of pyrazines was favored by using glucose and whey protein while furanmethanol and maltol formation was positively influenced by using milk protein.

Oppositional effects regarding sugar and protein concentrations have been found for 2,4-decadienal, representing a chemical compound derived by the autoxidation of fatty acids, providing typical off-flavor characteristics at higher concentration. Its formation was favored at increasing sugar contents and reduced by increasing protein concentrations. Proceeding from the assumption that 2,4-decadienal follows a different chemical pathway than pyrazines, furanmethanol and maltol it was found that those pathways are differently influenced by protein and sugar.

Acid formation was found to be dependent on the kind of sugar and protein used as well as on their concentration. By adding glucose syrup, acid formation reached its maximum at 4% followed by a distinct decrease. The same effect was found for butyric acid using glucose, while a minimum concentration at 4% glucose was detected for pentanoic acid and hexanoic acid. Increasing whey protein contents led to decreasing acid concentrations whereas the use of 2% milk protein maximized the concentrations of butyric and pentanoic acid. Hexanoic acid made an exception by showing consistently increasing concentrations at high milk protein concentrations.

The addition of different single amino acids as potential flavor precursors showed similarities in how they influence the formation of the examined flavor compounds. In general, the results were comparable to those found for protein. Differences were found in the intensity in which different amino acids affect the formation of the selected flavor compounds. Valine and proline showed the highest potential in reducing the amounts of 2,4-decadienal and acids whereas the highest levels of pyrazines were found using leucine. However, neither did one of the examined amino acids come along with definite advantages over the other amino acids nor were the detected variances distinct enough, compared to the reference sample, to justify their application.

Color measurement showed significant influences of all adjustable parameters, i.e. protein, sugar, and barrel temperature on product lightness. A quadratic influence was found for the barrel temperature (140°C – 180°C), resulting in maximum darkening at different temperature. This temperature depended on the protein-sugar composition used. A maximum darkening was reached at lower temperatures using milk protein, compared to samples containing whey protein. The addition of protein led to significant increasing product darkening due to Maillard type browning reactions. No differences could be found for the type of protein used. The addition of sugar resulted also in a significantly increasing color formation which was found to be more intense compared to protein. Thereby, more intense coloration was found using glucose compared to glucose syrup. A coherence regarding product lightness and the formation of specific flavor compounds could not be found.

This study demonstrated that the formation of flavor compounds and color development could be modulated by the tested parameters. Furthermore it could be shown that flavor

formation as well as coloration can be differently influenced depending on the kind of used additives, i.e. sugar, protein, and amino acid.

Zusammenfassung

Das Ziel der vorliegenden Promotion war die Untersuchung des Einflusses von Temperatur und spezifischer Prekursoren der Maillardreaktion auf die Aromastoffbildung und die Farbentwicklung in stärkehaltigen Extrudaten. Dazu wurden die funktionellen Beziehungen von Prozess-, System- und Produktparametern betrachtet.

Mischungen aus Weizenstärke und Protein wurde in einem gegenläufigen Zweiwellenextruder mit einem Scheckendurchmesser von 44 mm unter Zugabe von in Wasser gelöstem Zucker und einzelnen Aminosäuren bei verschiedenen Gehäusetemperaturen extrudiert. Für jede der untersuchten Protein-Zucker-Mischungen wurde ein 3^3-Faktorenversuchsplan mit den nachfolgenden Einstellungen verwendet.

i) Gehäusetemperatur 140°C, 160°C und 180°C

ii) Proteingehalt der Mischung 1,3%, 2,0% und 2,6%

iii) Zuckergehalt der Mischung 2%, 4% und 6%

Um die Zahl der möglichen Einflussgrößen zu reduzieren und eine weitestgehend gleichbleibende Verweilzeit zu erreichen, wurden Schneckendrehzahl sowie Extrusionsfeuchte und Gesamtmassenstrom nicht variiert.

Hinsichtlich der gemessenen Systemgrößen Düsentemperatur, Düsendruck und spezifisch mechanischer Energieeinleitung (SME) zeigten Protein, Zucker und Temperatur unterschiedlich starke Einflüsse. Die SME war der einzige Systemparameter für den ein statistisch signifikanter Einfluss auf die Aromastoffentwicklung festgestellt werden konnte. Ein Anstieg führte zu einer erhöhten Konzentration der aromaaktiven Pyrazine und des aromaaktiven Furanmethanols. Die statistische Auswertung zeigte für keine der untersuchten Aromastoffe eine signifikante Beeinflussung im Bereich des gemessenen Produkttemperatur- beziehungsweise Produktdruckbereiches.

Grundlage der Bewertung des Einflusses von Protein, Zucker und Temperatur sowie der in einer separaten Versuchsreihe untersuchten Aminosäuren auf die Aromaentwicklung waren die Konzentrationen ausgewählter Aromastoffe nach Auswertung von GC/MS-Analysen. Die Quantifizierung erfolgte hierbei mit Hilfe der Stable Isotope Dilution Analysis (SIDA) beziehungsweise durch die Verwendung von Trimethylpyrazin als internen Standard für die selektive Quantifizierung der Pyrazine. Proteine, Zucker und Temperatur zeigten hierbei unterschiedliche Einflüsse auf die Bildung verschiedener Aromastoffe. Es konnte gezeigt werden, dass die im Rahmen dieser Studie untersuchten Aromastoffe, welche für einen gewünschten cerealen Röstgeschmack verantwortlich sind, ähnlich beeinflusst werden. Hierzu zählten neben Pyrazinen auch Furanmethanol und Maltol. Bei allen zeigten sich zunehmende Konzentrationen mit steigenden Gehäu-

setemperaturen und Proteingehalten. Im Gegensatz dazu führten erhöhte Zuckergehalte zu sinkenden Konzentrationen an 2,5-Dimethylpyrazin, Furanmethanol und Maltol. Lediglich Methylpyrazin zeigte steigende Werte bis 4 % Zucker, gefolgt von einer Konzentrationsabnahme. Neben ihren prozentualen Anteilen im Endprodukt konnte ein deutlicher Einfluss der Art von Protein und Zucker festgestellt werden. Während die Verwendung von Glucose und Molkenprotein die Bildung der untersuchten Pyrazine begünstigte, zeigten Furanmethanol und Maltol höchste Konzentration in Proben welche unter Zugabe von Milchprotein hergestellt wurden.

Gegensätzliche Effekte wurden für die Bildung von 2,4-Decadienal festgestellt, einer während der Autoxidation von Fettsäuren gebildete Verbindung mit negativer Aromacharakteristik. Es konnte gezeigt werden, dass die Bildung stark durch steigende Zuckerkonzentrationen begünstigt wird, während steigende Proteingehalte zu Abnahme führen.

Die Bildung der untersuchten Säuren (Butansäure, Pentansäure und Hexansäure) war sowohl abhängig von der Konzentration als auch von der Art des Zuckers beziehungsweise Proteins. Die Zugabe von 4 % Glucosesirup führte hierbei zu maximalen Konzentrationen aller untersuchten Säuren, während dieser Effekt bei Verwendung von 4 % Glucose nur für Butansäure beobachtet werden konnte. Die Verwendung von Molkenprotein führte zu sinkenden Konzentrationen aller Säuren, wohingegen die Verwendung von Milchprotein zu Anstiegen führte.

Die verschiedenen Aminosäuren zeigten grundsätzliche Ähnlichkeiten in der Art und Weise wie sie die Bildung der untersuchten Aromastoffen beeinflussten. Diese war weitestgehend mit dem der Proteine vergleichbar. Untereinander zeigten sich hingegen Unterschiede in der Intensität der Einflüsse. Während durch die Verwendung von Valin und Prolin die Bildung von 2,4-Decadienal am effektivsten gehemmt werden konnte, zeigte Leucin den positivsten Einfluss auf die Pyrazinbildung. Ein deutlicher Vorteil einer der verwendeten Aminosäuren gegenüber anderen Aminosäuren oder der Verwendung von Protein konnte jedoch nicht gezeigt werden.

Die durchgeführten Farbmessungen zeigten einen signifikanten Einfluss aller Prozessparameter auf die Helligkeit der Extrudate. Für die Temperatur im Untersuchungsbereich von 140°C bis 180°C Gehäusetemperature konnte hierbei ein quadratischer Einfluss festgestellt werden. Abhängig von der verwendeten Protein-Zucker-Kombination zeigte sich eine maximale Farbintensität bei unterschiedlichen Temperaturen. Die Verwendung von Molkenproteinen führte hierbei erst bei höheren Temperaturen zu einer maximalen Bräunung im Vergleich zu Milchprotein. Sowohl Protein als auch Zucker beeinflussten die Farbbildung, wobei ein stärkerer Einfluss des Zuckers festgestellt wurde. Im Gegensatz zu den untersuchten Proteinen zeigte sich zusätzlich eine deutliche Abhängigkeit der Intensität der Farbstoffbildung von der Art des verwendeten Zu-

ckers. Die Verwendung von Glucose führte hierbei zu stärkeren Bräunungsreaktionen im Vergleich zu Glucose Sirup. Ein Zusammenhanges zwischen der Produkthelligkeit und der Bildung spezifischer Aromastoffe konnte nicht festgestellt werden.

Durch die vorliegende Studie konnte demonstriert werden, dass sich mit Hilfe der untersuchten Parameter Temperatur, Protein und Zucker die Bildung von Aroma- und Farbstoffen modulieren lässt. Des Weiteren konnte gezeigt werden, dass sich sowohl die Aromabildung als auch die Farbgebung verschiedenartig durch die Art des gewählten Zusatzes (Zucker, Protein, Aminosäure) beeinflussen lassen.

1 Introduction and Objectives

1.1 Introduction

Extrusion cooking is a modern process, which has become widely used in the manufacture of breakfast cereals and snack food. Compared with traditional processes, like baking, extrusion cooking combines multiple process operations such as mixing, cooking, and texturing in a single process unit. This results in very short processing times, small production spaces, and low energy consumption for the production of specific end products, like breakfast cereals.

The major shortcoming experienced with extrusion cooked cereal flours is the lack of flavor generation in the end products. This is due to the short cooking time and low temperature which the processed raw material encounters during extrusion. This limits the extent of chemical reactions of the raw material components which are responsible for flavor compound formation. In addition, flavor compounds are lost to the atmosphere as the product exits the extruder. This loss of flavor is a major concern as especially the volatile aroma components are regarded as the most important parameters of food flavor quality.

The formation of flavor compounds in conventionally processed as well as in extruded cereals is attributed to a series of reactions, with the Maillard reaction and degradation of lipids being the most prominent. However, the most important flavor compounds, providing typical cereal, roasted, toasted and caramel-like flavor impressions are generated during the Maillard reaction. For this, it requires the presence of reducing sugars and free amino groups to form such important flavor compounds as pyrazines, aldehydes, or pyrroles. These reactions are favored by elevated temperature and intermediate moisture, typical for extrusion cooking [1].

Over the past years, many studies on extrusion cooking were carried out which aimed at the examination of the influence of different cereal formulations on extrusion behavior, extrudate properties, and nutritional and physical attributes of cereal products [2]. However, no specific studies have been carried out as yet on the formation of flavor compounds during extrusion cooking in the presence of added protein and sugar as potential reactants for flavor generation.

Several studies have been addressed to how to influence Maillard reaction driven flavor formation by the addition of potential flavor precursors such as reducing sugar and amino sources or by changing process parameters. Many were carried out on liquid model systems, examining the influences of temperature, pH, selected sugars, and single

amino acids on the formation of flavor compounds such as pyrazines, derived from the Maillard reaction [3, 4, 5, 6].

With regard to extrusion processing, most studies focused on the influence of process parameters such as temperature and moisture on flavor formation or on changes in color, shape, texture, mouth-coating characteristics, and acceptability [7, 8].

The effect of single amino acids and selected reducing sugars on flavor compound formation and aroma characteristics have been intensively studied as well. Thereby it was found that each of them have significant influences on flavor formation. Briefly, amino acids as well as reducing sugars favor the formation of desired components mainly affected by the type of the used amino acid and the chemical structure of the sugar.

However, less is reported about the use of complex proteins as potential precursors of flavor generation. This is surprising since proteins are always present in the raw material used for this type of products and have also become important recipe ingredients in extruded food as a consequence of their availability at reasonable cost and their functional and nutritional values. Furthermore, they represent an excellent source of amino groups, necessary for the Maillard reaction [9]. *Solina* et al. examined the influence of soy protein and vegetable protein on the volatile components of extruded wheat starch [10]. To date, nothing has been reported about the interactive influences of complex proteins and different types of sugar on flavor development in dependence on extrusion temperature.

1.2 Objectives

The main aim of the dissertation was to use a recipe model system consisting of wheat starch, milk proteins, reducing sugars, and single amino acids for the examination of the influence of the recipe components and the extrusion temperature on the development of selected flavor compounds in the extruded end product.

In this study, dried milk powder from defatted milk as a source of complex milk protein and whey protein isolate were chosen to examine differences in their ability to influence the generation of flavor compounds. Both represent excellent amino group sources and are widely used as ingredients in the food industry. As for the sugar, the effect of glucose syrup was compared to that of glucose, as a pure reducing sugar.

These substances were added to wheat starch in different combinations and concentrations and subsequently extruded at different temperatures. To reduce the experimental complexity, 3^3 factorial designs were used, leaving the barrel temperature and the concentration of the used proteins and sugars as changeable parameters. Several statistical means were used to elucidate the interactions.

Regarding the flavor formation, eight selected important flavor compounds representing different chemical classes were analyzed. The aim was to get a general overview on how the changed process parameters influence the final product parameters, i.e. the flavor formation. In terms of a precious evaluation, two different techniques in sample preparation were examined. Therefore, the widely used headspace analysis was compared to a complex extraction-distillation method regarding their differences in detecting flavor compounds. Beside a qualitative analysis of the generated aroma compounds, their quantification was of high interest. Quantification by stable isotope dilution analysis was carried out on selected compounds to carve out its advantages in obtaining accurate results compared to those obtained by using common internal standards. The aim was to look for a possible application in conventional product control processes.

During extrusion cooking, four single amino acids were added in different amounts to a mixture of wheat starch and 4% glucose to evaluate their influence on flavor formation.

Additionally, color formation during extrusion processing, its measuring, and the examination of its possible applicability to the process control regarding flavor formation was the aim of this study. Since it is known that flavor formation during the Maillard reaction comes along with non-enzymatic browning, the effect of used sugars and proteins on resulting product lightness was analyzed and evaluated. The goal was to look for coherences between color formation and the formation of single flavor compounds.

2 State of the Art

2.1 Extrusion Cooking

Food extrusion has been practiced for over 60 years. While the first extruders were used as mixing and forming units for the production of pasta and ready to eat (RTE) cereal pellets, the first single-screw cooking extruders were developed in the 1940's to create puffed snacks from cereal flours. Modern extruders are considered to be high-temperature short-time (HTST) bioreactors which transform a wide variety of ingredients in intermediate and finished products. Several characteristics make modern extruders interesting for food processing (**Table 1**) [11].

Table 1: Characteristics of modern extrusion cooking

- Continuous high-throughput processing
- Energy efficiency
- Processing of relatively dry viscous materials
- Improved textural and flavor characteristics of food
- Use of unconventional ingredients

Source: Harper, J.M. (1981) [11]

Today, many food or feed products are produced by extrusion cooking technology. Breakfast cereals, snacks, pregelatinized flour and starch, pet food and animal feedstuff are some examples [12, 13].

Expanded extrusion products have gained their market mainly because of their pleasing crispy texture. To create such a puffed product, the starch and water containing ingredients are heated up inside the extruder by external heating of the barrel and by the mechanical energy, required to turn the screw, which is partly converted into heat. The heat raises the temperature of the mixture up to 180°C or more if necessary. The pressure drop at the die exit causes puffing to occur when the high-temperature water, entrapped in the gelatinized product rapidly evaporates [11, 14, 15, 16].

Regarding flavor formation, according to *Harper* et al. [11], extrusion tends to be limiting because of its short-time treatment and the loss of volatiles which are being flashed off at the die. However, flavor formation during extrusion cooking is complex and depends on several parameters. The use of extrusion cooking with regard on flavor formation has been shown by *Meuser* et al. [17] who integrated HTST extrusion cooking into

the malting process during beer production. The obtained extrudates were characterized by high yields of flavor compounds identically to those generated by conventional malting. It was found that the flavor compounds as well as the intense browning are mostly generated during Maillard reaction, positively influenced by increasing temperature and SME [18, 19, 20].

A closer look on flavor formation during extrusion cooking is given in chapter 2.3.1.

2.2 Expanded Ready to Eat Cereals

Ready to eat cereals (RTE) are either produced by gun puffing or extrusion cooking. These processes are used to convert raw, dense grain or milled fractions of these into friable, crisp, or chewable products, suitable as human food.

2.2.1 Puffed Cereals

One major group of puffed cereals are gun-puffed whole grain cereals. According to *Fast* et al. [21], whole rice or wheat kernels are predominantly processed this way. Two things are necessary for the grain to puff. It must be cooked, and a large, sudden pressure drop must occur in the atmosphere surrounding the grain which forces the liquid to convert into steam instantly. Today, the puffing process mostly takes place in so called single- or multiple-shot guns which are operated automatically and partly continuously. The gun(s) is (are) loaded with grain and steam is injected into the gun body at 14 bar (200 psi). By doing so, the necessary time to transfer the heat into the grain to cook and condition it for puffing could be reduced to as low as 90 sec compared to 9-12 min with traditional batch puffing single-shot gun.

The starchy matter of other cereal grains, such as oats or corn can be puffed as well. For this, milled products of these cereals are used. The cooking process, necessary to gelatinize the starch doesn't take place in the shot gun but in an extruder. That is why this group is called extruded gun puffed cereals. The basic dry materials like flour and grits and a solution made of water and, depending on the final product, sugar, salt, malt, colors and flavors is fed into a cooking extruder in where it is gelatinized. After the cooked dough exits the extruder, it is fed to a forming extruder which is usually controlled at noncooking temperatures below 70°C. After drying and tempering to a moisture content of 9-12% the shaped pieces are then gun puffed as described above.

Another group of puffed cereals are the oven puffed. These are almost exclusively made from rice or corn since they puff easily in the presence of elevated heat and correct moisture content, whereas wheat and oats do not. After several steps of preconditioning like cooking, drying and bumping (slightly flattening of the kernels by running through

flaking rolls) the grain is puffed in special rotary flake-toasting ovens or other fluid-bed oven, characterized by extremely high temperatures (288-343°C).

The process of oven puffing as well as gun puffing is followed by cooling and screening. If necessary the final products can be fortified with vitamins and flavors or treated with antioxidants to preserve freshness [21].

2.2.2 Extruded Expanded Cereals

Direct puffed snacks and cereals made by extrusion process are classified as a second generation snack. As well as conventional puffed products they are usually low in bulk density and are often marketed as high-fiber, low-calorie, high-protein and nutritional products [22, 23].

In contrast to conventional puffed cereals, the whole process of cooking and expanding happens in a single unit, the extruder. This technology operates on a continuous basis, is applicable for all kinds of grain and uses flour or meal rather than whole or broken kernels. Once the formulation has received its cooking, it is pressed through the holes of die at the end of the extruder which controls the shape of the product. By leaving the zone of elevated temperature and pressure inside the extruder, the product expands when moisture converts into steam. The cutting of the expanded or expanding product is usually done by a knife rotating on the outer face of the die.

As well as the conventional processes, the production of extruded expanded products is followed by drying, cooling and fortification steps. Especially flavoring, coloring and sugar coating allow the creation of products for various tastes.

The advantages of this process over conventional techniques are both economic and practical in terms of a reduction in space, time and energy [14, 21].

2.3 Flavor

Flavor in principle is the overall sensation, provided by the interaction of taste, odor and textural feeling, when food is consumed. Flavor results from compounds that are divided in two broad classes. Those responsible for taste are generally nonvolatile at room temperature. Therefore, they interact only with taste receptors located in the taste buds of the tongue. Compounds responsible for the odor are often designated as aroma substances. They are volatile and are perceived by the odor receptor sites of the smell organ, i.e. the olfactory tissue of the nasal cavity [24].

Flavor particularly influences acceptance by consumers, and volatile aroma components generally are regarded as the most important parameters of food flavor quality.

Specific flavor compounds can be grouped rather simply as being derived from environmental, metabolic, microbial, chemical, or thermal sources. Since flavor development in extruded cereals is the basis of this thesis, the main factor contributing to flavor formation is temperature, and thus thermally produced flavor compounds are of major concern.

2.3.1 Flavor in Extruded Expanded Cereals

The number of flavor compounds, present in various food flavor systems totals into the hundreds and thousands. However, the presence of a specific flavor associated compound does not mean that it makes a significant contribution to the characteristic flavor of a specific food.

Even if progress in instrumental analysis, particularly high resolution gas chromatography and mass spectrometry, has shown that the volatile fraction of heated cereals consists of a multiplicity of compounds only a few are important [23, 25, 26].

Grosch and *Schieberle* [27] pointed out that only a small number of volatiles are of significance in determining the flavor of heated cereals which are characterized by a roasted, toasted, and nutty flavor impression

Most extruded cereals are primarily composed of carbohydrates such as starch and sugars, protein, lipids, and moisture. These ingredients serve as major precursors of the thermally produced flavor compounds.

During extrusion the most significant reactions in heat induced flavor compound formation are nonenzymatic browning reactions typified by caramelization and Maillard reaction. Those are the same reactions as they appear during traditional processes such as baking or frying. As the name implies, this reactions usually produce product browning. From a flavor point of view nonenzymatic browning is a highly desirable reaction occurring during extrusion since important compounds are produced. However, the rate of chemical reaction or rather flavor generation is much lower due to a shorter residence time compared to baking and a much lower temperature compared to frying.

The wide use of extruder in the production of foodstuff has led to a number of reviews detailing the effects that extrusion has on odor generation and odor retention in extruded products. Some recent studies in this field have led to a better understanding of the influence of different extruder conditions on flavor development. *Bredie* et al. [26] and *Parker* et al. [14] demonstrated the influence of high barrel temperatures and low moisture contents on an increasing yield of Maillard reaction products, so important in the development of baked and roasted odors, using maize, wheat, and oat flours.

Bredie et al. [28], *Ames* et al. [29], *Jusino* et al. [30], and others independently examined the influence of the pH and found that levels of pyrazines and other flavor com-

pounds, beside furans, generally increase with pH which is in contrast to a study by *Bonvehi* et al. [31] who stated that higher pH values let to lower concentration of aroma but higher presence of brown color.

No studies could be found detailing the effect of screw speed variations on flavor formation. *Hsieh* et al. studied the effects of screw speed on processing and product variables such as die pressure, SME, and expansion index but it remains unclear if the examined changes may have an influence on the formation of aroma compounds [12]. The general influence of process and system parameter on chemical reactions during extrusion cooking has been studied by *Gimmler*, focusing not on flavor compound formation but on the derivatization of starch during extrusion cooking [32]. He found higher rates of chemical reactions at longer residence times and elevated SME inputs. Similar examinations were made by *Jusino* et al. [30] and *Huang* et al. [33]. They investigated the formation of pyrazines in solid model systems and found it favored at high temperature and long residence time.

The gained information is of high interest but partially conferrable on the present study only. This is due to the fact that the solid model studies mentioned, investigated the pyrazine formation over a longer period of time and lower temperatures as it is possible during extrusion cooking. On the other hand, *Gimmler* used the process of extrusion cooking but analyzed its influence on a chemical reaction that would happen even under non processing conditions. All of these studies showed that chemical reactions are influenced not only by the energy input, but by time as well.

Beside the variation of process and system parameters, different studies examined the effect of specific precursors on flavor formation. The influence of single amino acids and selected reducing sugars on flavor compound formation and aroma characteristics have been studied most intensively. Thereby it was found that both have significant influences on flavor formation. Especially amino acids favored the formation of desired components whereas the latter strongly depended on the type of the used amino acid. [20, 29, 44, 46, 49, 61, 62, 104]

However, less is reported about the use of complex proteins as potential flavor precursor. Proteins have become important ingredients in extruded food as a consequence of their availability at reasonable cost and demonstrated functional and nutritional values. Their influence on nutritional, physiological and physical properties of extruded cereal products have been studied by *Köhler* [34]. Furthermore, proteins represent an excellent source of amino groups, necessary within the Maillard reaction [35]. *Solina* et al. examined the influence of soy protein and vegetable protein on the volatile components of extruded wheat starch [25, 36]. But still, nothing has been reported yet about the interactive influences of complex proteins and different types of sugar at varying extrusion temperatures on flavor development.

Summarizing it can be said that by extrusion cooking important flavor compounds can be generated by Maillard reactions. This flavor generation is limited because of the short residence time of the mass in the extruder barrel. The benefit of specific Maillard reaction precursors was primarily investigated by using single amino acids and selected sugars. Cognitions about the influence of complex proteins and their interactions with sugars at different temperatures are still missing.

2.3.2 Flavor Development by Nonenzymatic Browning Reactions

There are three basic nonenzymatic browning reactions that cause flavor in heated food systems. Because of the high temperature, the short processing time and the presence of reducing sugars and amino groups, the so called Maillard reaction is the most important in the production of extruded expanded cereals. Therefore it will be described more preciously while there will be just a short overlook on the other two.

2.3.2.1 Pyrolysis

Pyrolysis is simply scorching, from the Greek “pyro”-burning, and involves the total loss of water from the sugar molecule and the braking of carbon-carbon linkages, i.e. the “destruction” of the sugar molecule. It requires higher temperatures as given in processing cereals and results in burnt and inedible flavors as well as in chemical compounds that showed a highly mutagenic effect in microbial tests [24].

2.3.2.2 Caramelization

Beside Maillard reaction and enzymatic browning, the caramelization represents the most important type of browning process appearing during heating and roasting of carbohydrate-rich food. It is a transformation of reducing sugars alone in a concentrated solution through so called anhydro sugars. In this reaction sugars of a low degree of polymerization lose water molecules from their structure through 1:2 and 2:3-enolisation followed by polymerization steps.

The whole process of caramelization requires high temperatures and can be directed more toward aroma formation or more toward brown pigment accumulation. Heating glucose syrup with sulfuric acid in the presence of ammonia as a source of nitrogen provides intensively colored polymers. On the other hand, heating the syrup in a buffered solution enhances molecular fragmentation and thereby formation of aroma substances [24].

In general, the temperature required for caramelization depends on the type of sugar. Initial caramelization temperatures of common sugars are shown in **Table 2**. *Brands* et al. [37] compared a fructose-casein and glucose-casein model system. They verified that browning depends strongly on the temperature and found that after an induction time during which no browning is detected, fructose browns more quickly than

glucose. Due to differences in reaction behavior between glucose and fructose it remained unclear if browning is attributed to different caramelization temperatures or to differences in reactivity within the occurring Maillard reaction.

However, it is undoubted that caramelization takes place during extrusion processing, contributing to color and flavor [24, 38].

Table 2: Initial caramelization temperatures of common sugars

Sugar	Temperature
Fructose	110°C
Galactose	160°C
Glucose	160°C
Maltose	180°C
Saccharose	160°C

Source: Food-Info.net (2008) [38]

2.3.2.3 Maillard Reaction

The Maillard reaction is named after the chemist *Louis-Camille Maillard* .

It represents a chemical reaction caused by the condensation of a nucleophilic amino group e.g. of an amino acid and the carbonyl group of a reducing sugar.

The Maillard reaction is of utmost importance for quality of foods, more in particular for heated foods. It induces browning of foods, has an effect on nutritive value, can have toxicological implications (such as the formation of acrylamide, see chapter 2.3.5), can produce antioxidative components (reductones) and it has, most important, a large effect on flavor. For instance, the Maillard reaction is responsible for the desired aroma formation accompanying such important processes like cooking, baking, roasting or frying. It is equally significant for the generation of off-flavors in food during storage or on heat treatment for the purpose of pasteurization, sterilization and roasting. It can also produce partially desired bitter substances (coffee).

Beside the development of important volatile aroma substances, the Maillard reaction is well known for generating brown pigments, known as melanoidins which contain variable amounts of nitrogen. They are desired in baking and roasting, but not in food which have a typical weak or other color of their own (condensed milk, tomato soup).

In the process, hundreds of different flavor compounds are created. These compounds in turn break down to form yet more new flavor compounds, causing a chain reaction. A

short overlook on the Maillard reaction regarding generated flavor compounds might be given by **Figure 1** [39, 40].

Each type of food has a distinctive set of flavor compounds that are formed during the Maillard reaction. Selected flavor compounds, important for generating cereal like flavor impressions, are elucidated more detailed in chapter 2.3.4.

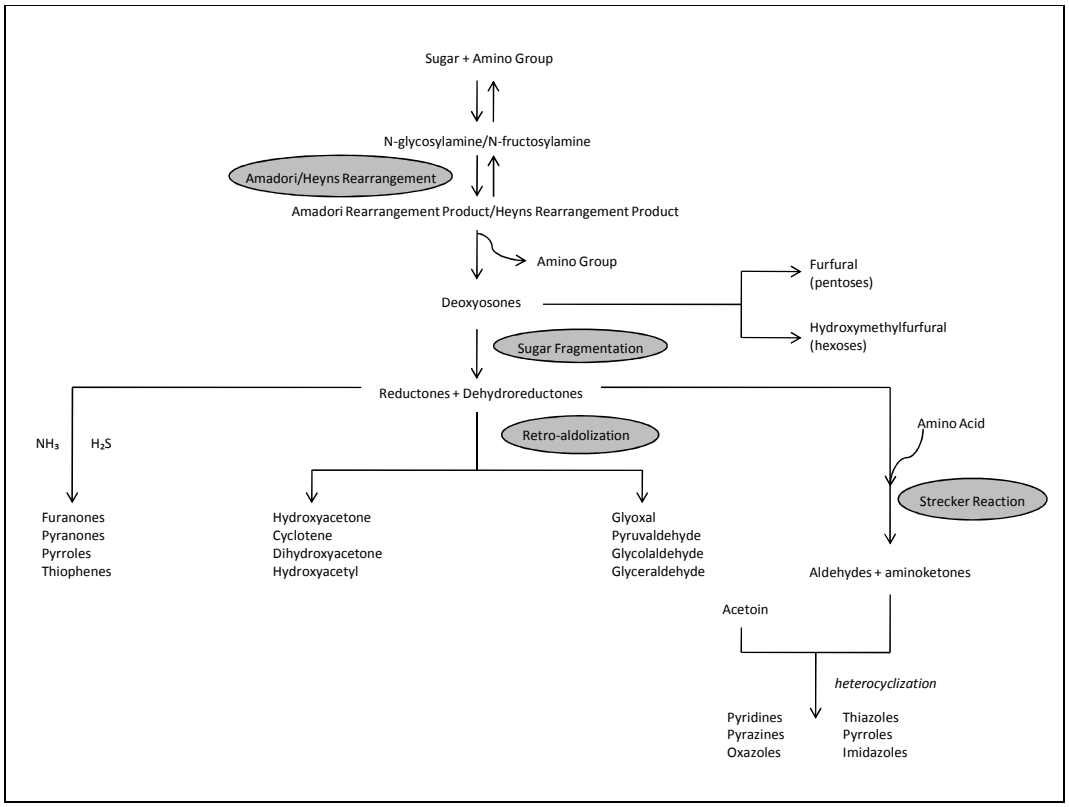


Figure 1: General overview of the Maillard reaction showing flavor compounds as end products.

Source: Ikan, R. (1996) [41]

2.3.2.3.1 Chemical Outline of the Maillard Reaction

Most of the literature is subdividing the Maillard reaction in three basic phases. An outline of the Maillard reaction, adapted from the *Acta Tabacaria Sinica* (2004)[42], matching the following explanations is given in **Figure 2**.

The initial stage starts with the condensation of the carbonyl group of a reducing sugar with a free amino group of a protein or an amino acid, which loses a molecule of water to form N-substituted glycosamine. (Step A). This is unstable and undergoes, in the case of an aldose sugar, the Amadori rearrangement to form an Amadori product or in the case of a ketose sugar, the Heyns rearrangement to form a Heyns product (Step B).

These products can then react further in three ways in the second phase.

One is the simply further dehydration by the loss of two water molecules into reductones and dehydro reductones (Step C). These are essentially "caramel" products and in their reduced state powerful antioxidants.

A second is the production of short chain hydrolytic fission products such as diacetyl, acetol, pyruvaldehyde, etc. (Step D). These than undergo "Strecker degradation" with amino acids to aldehydes (Step E) and by condensation to aldols, or they may react in the absence of amino compounds, to give aldols and high molecular weight, nitrogen-free polymers (Step F).

A third path is the Schiff's base-furfural path. This involves the loss of three water molecules (Step C), then the reaction with amino acids and water.

All these products react further in the final Maillard reaction with amino acids to form brown nitrogenous polymers and copolymers called melanoidins (Step G). These can be off flavors (bitter), off aromas (burnt, onion, solvent, rancid, sweaty, cabbage) or positive aromas (malty, bread crust-like, caramel, coffee, roasted).

Step H illustrates a direct route to fission products from N-substituted glycosylamines, without the formation of an Amadori rearrangement product.

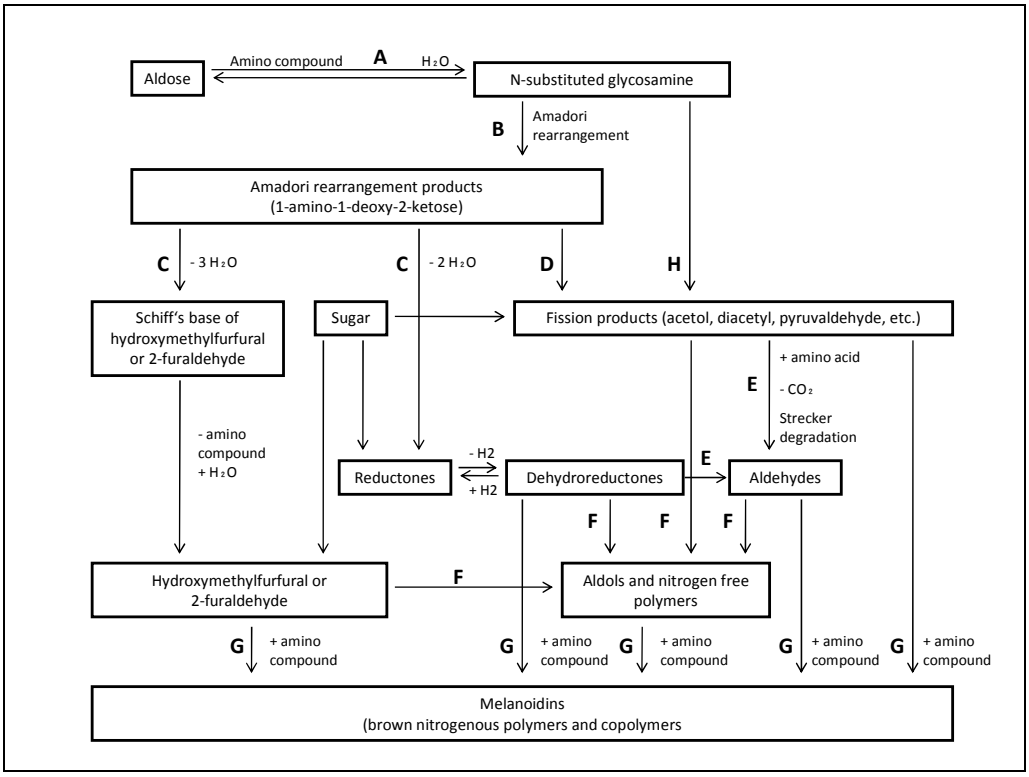


Figure 2: Outline of the chemical reactions occurring during the Maillard reaction.

Source: Acta Tabacaria Sinica, China Tobacco Society, Bejing/China, 2004

2.3.3 Flavor Precursors

Since the Maillard reaction is the most important chemical reaction producing flavor compounds during extrusion processing, the addition of reactive flavor precursors such as reducing sugars, proteins and single amino acids can help to increase the amount of flavor volatiles or to change the aroma profile. It is very important to know about the influence of different proteins and sugars since they determine the type of flavor components formed during extrusion cooking while process parameters such as temperature, pH, time and water contents influence the kinetics [43].

Several studies are published, examining the influence of different sugars, proteins, and amino acids alone or in combination with flavor development. *Solina* et al. [25] extruded wheat starch/glucose mixtures with and without acid-hydrolysed vegetable protein at 180°C and analyzed differences in flavor formation. They found aroma potent lipid oxidation products to appear in every sample but showed that Strecker aldehydes, important flavor components, as well as flavor precursors, are dominant components in samples containing added protein. It is even more important that pyrazines, responsible for desired roasted flavor impressions, appeared exclusively in samples containing both, glucose and protein. These results are confirmed by *Bredie* et al. [46] who extruded wheat flour and wheat starch adding the single amino acid cystein and the reducing sugars glucose and xylose. In this study it is shown that cysteine and sugar are important precursors for nutty/roasted, and popcorn aromas when extruding wheat flour and wheat starch. The natural occurring contents of fat and protein in wheat flour were also found of importance, as the formation of more desirable aromas was favored by these in extruded wheat flour compared to wheat starch.

2.3.3.1 Sugar

From the biochemical point of view there are two main groups of sugars, the reducing and the non-reducing sugars. Only a reducing sugar can act as a catalyst in the Maillard reaction since it. A reducing sugar is any sugar that, in basic solution, forms some aldehyde (aldose sugars) or ketone (ketose sugars). The reactive carbonyl group, terminal in aldose and linked to two other carbon atoms in ketose (**Figure 3**), allows the sugar to act as a reducing agent, important in the Maillard reaction. While no differences are reported between ketoses and aldoses regarding their influence on the Maillard reaction, it is well known that pentoses participate in the Maillard reaction at a greater rate than hexoses because of its shorter chain length [44, 45].

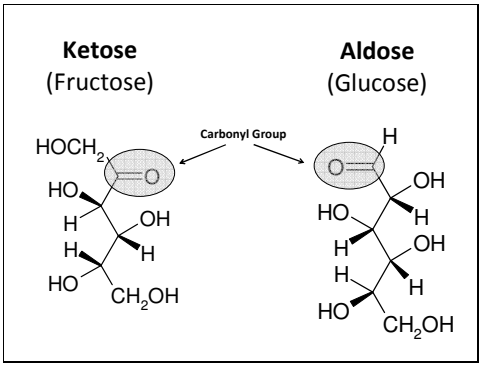


Figure 3: Carbonyl group location in ketoses and aldoses.

Well known reducing sugars are the pentoses xylose and ribose and the hexoses glucose and fructose as well as lactose and maltose, both disaccharides consisting of two hexoses.

Common table sugar, known as sucrose or saccharose is a disaccharide as well. It consists of one molecule glucose and one molecule fructose, joined by a glycosidic bond between carbon atom 1 of the glucose and carbon atom 2 of the fructose unit (**Figure 4**). Unlike most polysaccharides, the glycosidic bond is formed between the reducing ends of both glucose and fructose. Hence it contains no free atomeric carbon atom and is therefore classified as a non-reducing sugar, ineligible to enhance Maillard reaction processes.

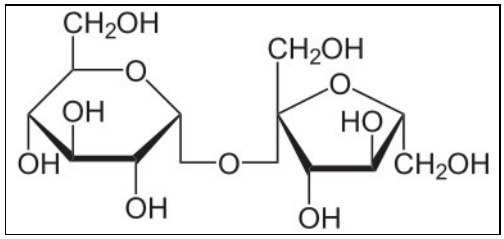


Figure 4: Saccharose molecule consisting of α-D-Glucose and β-D-Fructose.

Beside the advantage of being a cheap and therefore widely used sugar in the food industry, it is reported that glucose supports the creation of targeted cereal flavor impressions. In a sensory study, *Ames* et al. [44] established that extrudates made of wheat starch and cysteine in combination with glucose were more frequently described as “biscuit” and “nutty” whereas the use of xylose resulted in “meaty” flavor impressions. On the other hand *Bredie* et al. [46] stated that the nature of the added sugar (glucose vs. xylose) has only a small effect on sensory characteristics or rather on aroma generation. That underlines the difficulties in evaluating different sugars regarding their influence on the overall flavor of a final product even if it is undoubted that hexose sugars are

responsible for other flavor compounds than pentose sugars. For example, furfural is generated from pentose sugars whereas hydroxymethylfurfural is formed from hexose, both important intermediates in the Maillard reaction [24].

However, the relevance of sugar in flavor formation during the thermal treatment of food is undoubted and independently confirmed by numerous studies [20, 25, 26, 46, 62, 63].

2.3.3.2 Protein and Single Amino Acids

Beside a reducing sugar, free amino groups are necessary for creating flavor compounds via Maillard reaction. Free amino groups are naturally found in amino acids. An amino acid is a molecule that contains both amine and carboxyl functional groups (**Figure 5**). In biochemistry, this term refers to alpha-amino acids with the general formula $H_2NCHRCOOH$, where the R is a hydrogenorganic substituent. In the alpha amino acids, the amino and the carboxylate groups are attached to the same carbon, which is called the α-carbon. There are 20 proteinogenic alpha amino acids. They differ in their side chain (R), attached to the alpha carbon, and are the building blocks of all proteins. [47]

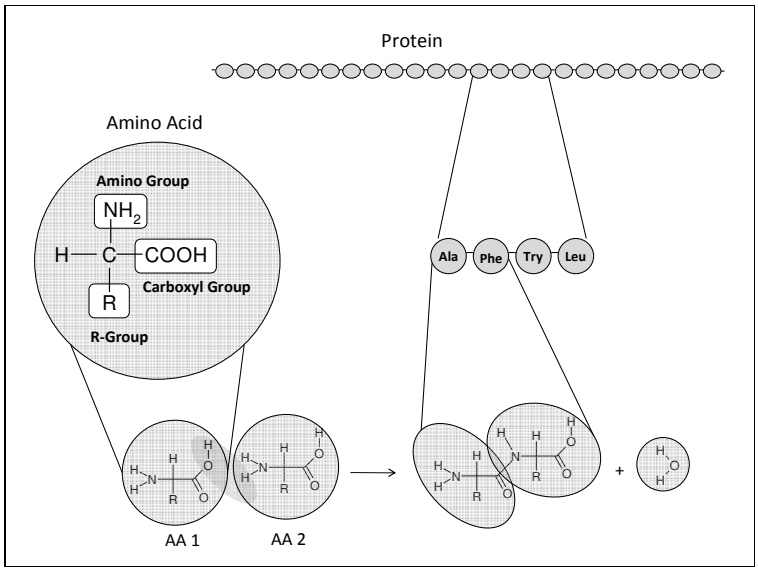


Figure 5: Amino acid and its functional groups as a part in the primary structure of a complex protein.

The effect of different amino acids on the formation of Maillard types of flavor compounds has been widely studied. However, most of the research on the formation of Maillard-based flavor compounds is on mixtures of sugar and single free amino acids, and hardly on sugar protein mixtures. Some examples are given by *van Boekel* [39] who

states that meat related flavor compounds are mainly sulfur containing compounds, derived from cysteine, while the amino acid proline gives rise to typical bread, rice and popcorn flavors. Pyrazines possess typical and desired cereal, roasted, and toasted flavors and are therefore meaningful flavor compounds in this study. *Ames* et al. [44] pictured the effect of amino acid on the generation of pyrazines in extruded starch model systems and figured out that the pyrazine level increases in the presence of all amino acids but cysteine, which enhanced the formation of sulfur compounds. Nevertheless cysteine and its flavor precursor qualities is investigated intensively because cysteine contains a nitrogen and a sulfur atom, necessary in heterocyclic flavor chemicals such as furans, pyrazines and thiazoles [48, 49, 50, 51, 52, 53, 54, 55, 56, 57].

With proteins, but in the absence of free amino acids, the Strecker reaction cannot take place, and this has consequences on flavor generation. Free amino acids may be generated during heating from proteins if hydrolysis occurs, but this will be limited during normal heat treatment of foods. At this, cooking extrusion could make an exception because of its high temperature treatment in combination with a subsequent drying/roasting process. *Bailey* et al. [45] added whey protein concentrate to corn meal at concentrations ranging from 0% to 20% and measured the flavor and color of the extrudate. They found that the production of pyrazines, furans, and other heterocyclics increased as the product temperature and concentration of whey protein, an excellent source of lysine and sulfur containing amino acids, was increased. Recently, *Wright* et al. [58] and *van Boekel* [63] confirmed that whey protein acts as a flavor precursor but found, that the high amounts of sulfur containing amino acids may cause off-flavors.

What remains in food systems without free amino acids is that sugar degradation products can react with the ε-amino groups of lysine residues, but also with other amino acid side chains, such as arginine and tryptophan. It is known that Maillard reaction is responsible for undesired flavor compounds in milk products, which hardly contain free amino acids. That proofs that the Maillard reaction can take place mainly via the lysine side chains, the major amino acid in milk.

2.3.4 Selected Flavor Compounds

2.3.4.1 Pyrazines

Pyrazines are heterocyclic, aromatic, organic compounds. The simplest representative is the pyrazine itself which is shown in **Figure 6**. Many different ones are known, at least 70 alkylpyrazines, consisting of only carbon, hydrogen, and nitrogen. Most often, pyrazines possess roasted, nutty, popcorn-like or green odors, low odor threshold values, and are responsible for typical food flavors. One often states their formation by the Strecker synthesis, part of the Maillard reaction [59, 60, 61].

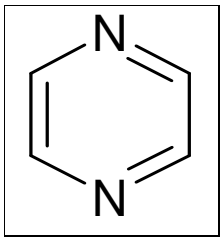


Figure 6: Pyrazine

Many studies were carried out, trying to examine the formation of pyrazines and their importance for the over-all flavor of starch containing food systems. Many of the pyrazines have been reported among the volatile products of heated, reduced-moisture systems or of extruded food systems. In a range of aqueous food systems, pyrazines have been reported as relatively minor reaction products whereas they were major components in reduced-moisture food systems such in extrusion. The influence of additional reducing sugar and amino acids is examined by *Farouk* et al. [62], who extruded wheat flour with glucose and different single amino acids. He showed that levels of pyrazines increased in all systems except the one containing cystein. This is undoubtedly accounted for in part by alternative pathways open to glucose degradation products in the cystein containing systems, due to the availability of highly reactive sulfur reactants deriving from cystein.

High temperature is very important in generating pyrazines, especially in conventional food systems without additional free amino acids. Proteins and peptides, natural components in food, consist of bounded amino acids, not available for the Strecker reaction as an essential part for the generation of heterocyclics such as pyrazines (as shown in **Figure 1**). As mentioned in 2.3.3.2, free amino acids may be generated during heating from proteins or peptides if hydrolysis occurs [63, 64].

2.3.4.2 Furanones / Pyranones

Furanones and pyranones are cyclic chemical compounds, containing an unsaturated fivemembered (furanones) or sixmembered (pyranones) ring with one oxygen atom and a ketone functional group (**Figure 7**).

Among the great number of products obtained from carbohydrate degradation, the furanones 3(2H)- and 2(5H)-furanone belong to the most striking aroma compounds. As well as maltol, the best know representative of the pyranones, they possess a caramel-like odor.

Additionally, maltol enhances the sweet taste of food, especially sweetness produced by sugars, and is able to mask bitterness.

Furanones and pyranones are found as secondary products of the Maillard reaction but *Grosch* et al. [24] and *Baltes* [47] describe other formation pathways such as aldol condensation, too. Furthermore they address difficulties in quantitative analysis of furanones due to their good solubility in water what makes them extractable from aqueous food only with poor yields.

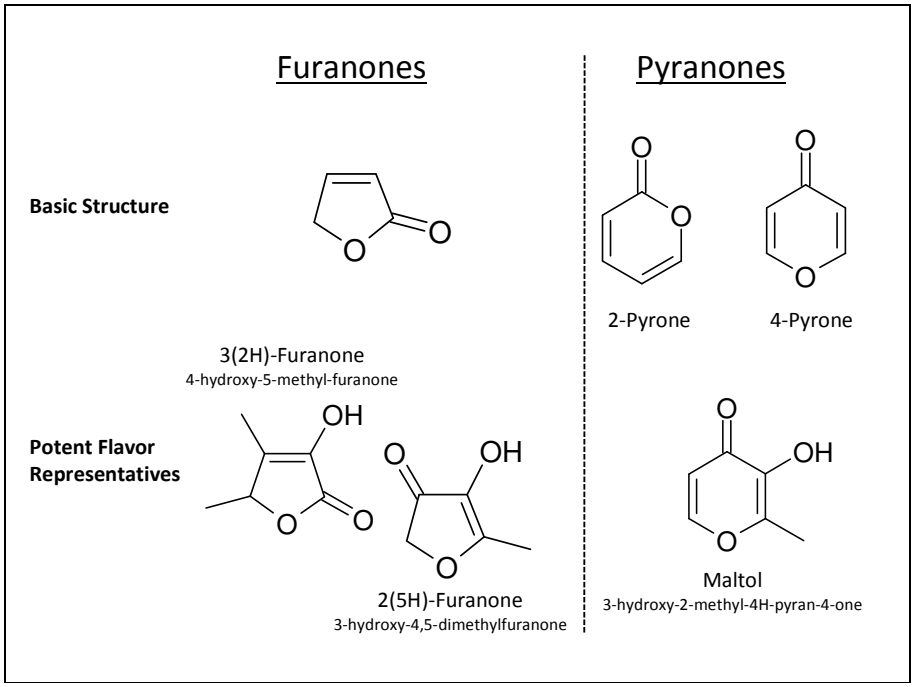


Figure 7: Basic structures and potent flavor representatives of furanones and pyranones.

2.3.4.3 Pyrroles / Pyridines

Pyrroles and pyridines are heterocyclic, aromatic, organic compounds. While pyrroles consist of a five-membered ring, pyridines contain a six-membered ring. Numerous pyrrole and pyridine derivates are found among the volatile compounds formed by heating of food. The structural feature shown in **Figure 8** is of special interest and appears to be required for roasted or cracker-like odor. The most potent representatives with a desired roasted aroma are 2-acetyl-1-pyrroline (white-bread crust, rice, popcorn, heated meat), 2-propionyl-1-pyrroline (popcorn, heated meat), 2-acetyltetrahydropyridine (white-bread crust, popcorn), and 2-acetylpyridine (white-bread crust). Independent studies announced 2-acetyl-1-pyrroline to be the outstanding flavor compound and a significant contributor to the aroma of several cereal products [27, 56, 65, 66, 67].

Grosch et al. [24] and *Schieberle* [68] showed in model experiments that 1-pyrroline, formed by the Strecker degradation of both proline and ornithine in the Maillard

reaction, act as the precursor of 2-acetyl-1-pyrroline as well as of 2-acetyltetrahydropyridine. *Suyama* et al. [69] presumes that the generation of pyridines may involve the condensation of aldehydes, ketones, or α,β-unsaturated carbonyl compounds with ammonia which is degraded from amino acids.

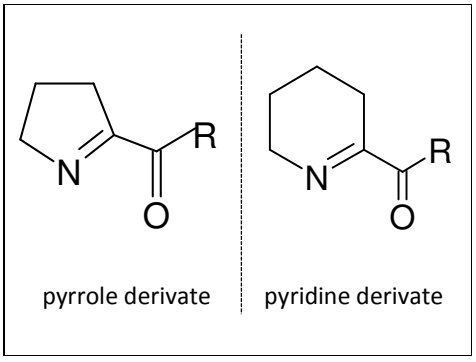


Figure 8: Basic structure of pyrroles and pyridines, required for roasted odor.

2.3.4.4 Aldehydes

Aldehydes are organic compounds containing a terminal carbonyl group. This functional group, which consists of a carbon atom bonded to a hydrogen atom and double-bonded to an oxygen atom (chemical formula O=CH-), is called the aldehyde group.

Aldehydes are potent flavor components in many foods with several flavor impressions. A short overlook on flavor characteristic are given in **Table 3**. They are generated by different pathways. A major group consists of the secondary products of lipid autoxidation. It is well known that aldehydes with exceptionally strong aromas can be released in food by autoxidation of some unsaturated fatty acids, even if they are present in low amounts. Due to their aroma characteristic and low threshold values, autoxidational generated aldehydes often contribute to off-flavors. It is to say, that autoxidation products are of less importance within this study since the analyzed samples contain only very small amounts of free fatty acids due to use of wheat starch and low levels of fat containing additives.

Furthermore and more importantly, aldehydes are formed in different ways during Maillard reaction. The most important one is the decomposition of amino acids by the Strecker degradation mechanism which generates some key odorants for toasted cereal flavor impressions such as 2- and 3-methylbutanal. Furfural and 5-hydroxymethylfurfural, both formed by sugar degradation in the Maillard reaction are further impact flavor compounds in extruded cereals [24].

Benzaldehyde, known for its typical bitter-almond odor, is a thermal reaction product as well. *Bruechert* et al. [70] explains its generation by thermal reactions of 2,4-decadienal and hexanal but other pathways are described in literature as well [71].

Table 3: Flavor impressions and formation pathways of selected aldehydes

Aldehyde	Flavor Impression	pathway
hexanal	grassy-flowery	lipid autoxidation
octanal	fatty	lipid autoxidation
nonanal	floral-fatty	lipid autoxidation
(E,E)-2,4-nonadienal	oily	lipid autoxidation
(E,E)-2,4-decadienal	fatty	lipid autoxidation
2-methylbutanal	malty	Strecker degradation
3-methylbutanal	malty	Strecker degradation
ethanal	sharp, penetrating, fruity	Strecker degradation
furfural	bread-like, caramelic	sugar degradation
5-hydroxymethylfurfural	sweet, hey-like, caramel	sugar degradation
benzaldehyde	bitter almond	differently

Source: Belitz, H.D. et al. (2004) [24], Arctander, S. (1969) [59]

2.3.5 Maillard Reaction and its Influence on Acrylamide Formation

Numerous studies have recently been performed on acrylamide and its formation in thermal processed carbohydrate-rich foods. It has caused worldwide concern because this compound has been classified as probably carcinogenic in humans. According to *Schlund*, World Health Organization (WHO) coordinator of food safety research, acrylamide probably has about the same potency as heterocyclic amines which caused cancer in animal tests. However, only little is known about potential health risks in humans and remains to be investigated.

More is known about its formation. *Mottram* et al. [72] reported that the Maillard-driven generation of flavor and color in thermally processed foods can - under particular conditions - be linked to the formation of acrylamide. Researches, addressing the question of precursor for acrylamide generation in the Maillard reaction pathway, suggest the naturally occurring amino acid L-asparagine as the major precursor when heated under low-

moisture conditions in the presence of reducing sugars or carbonyl compounds. This is accounted to the chemical structure of L-asparagine which already has an amide group attached to a chain of two carbon atoms. This knowledge is of high importance since L-asparagine is found to be a major amino acid in cereals, representing, for example, 14% of the total free amino acids in wheat flour [73].

Beside the Maillard-pathway, the possibility of pyrolytic acrylamide formation in baking processes is widely discussed. *Claus* et al. added purified wheat gluten, free of any reducing sugars, amino acids, and starch to bread roll dough to diminish the content of L-asparagine and reducing sugar. Nevertheless, he could show a correlation between the higher proportion of gluten and increased acrylamide content [74, 75].

In a model study, *Stadler* et al. measured the yields of acrylamide after Pyrolysis of N-glycoside, an early Maillard product and found the results comparable to those from amino-acid and reducing sugar precursors under the same conditions [76].

Both studies confirmed the assumption of acrylamide formation in heated foods independently from the presence of L-asparagine.

With regard to the present study, acrylamide and its formation were not investigated. However, based on the named literature it could be hypothesized, that under tested conditions an acryl amide formation that could raise concerns would be unlikely to occur. Neither severe time-temperature regimes as they are reported by *Claus* et al., necessary for pyrolysis, nor relevant precursors such as L-asparagine are used in this model study in significant amounts.

2.4 Flavor Analysis

Flavor analysis is carried out stepwise and can be subdivided in instrumental and sensory analysis. The main aspect of this thesis is to show the influence of temperature, sugars, and different amino sources on selected, well known flavor compounds in a starch based extrusion model system. Therefore, the focus was on detecting and quantifying those compounds.

2.4.1 Flavor Isolation and Concentration

There are several techniques in isolation and concentration of volatile compounds. One major group uses organic solvents such as ether for extraction or steam distillation while another rather simple technique use the headspace. Each of them comes along with specific advantages and disadvantages compared to others. That makes it hard to define the most suitable method which has to fit the particular bill [77].

In general, more compounds are found when organic solvent extraction or steam distillation methods are used for isolation than when headspace methods are used. However,

the use of solvent extraction or steam distillation allows the possibility of artifact formation or the introduction of impure solvents that increase the number of additional compounds that are identified later. *Folkes* and *Gramshaw* [78] isolated a total of 190 volatiles of white bread crust by ether-extraction followed by vacuum distillation while *Chang* et al. [79] identified "only" 63 volatile compounds in breads made from hard red winter wheat and hard white winter wheat by using the headspace.

In the isolation of aroma substances, foods which owe their aroma to the Maillard reaction should not be exposed to temperatures of more than 50°C to avoid accosted artifact formation or additionally formed compounds, i.e. thiol in the reduction of disulfides by reductones. Therefore the gentle extraction according to *Kutscher-Steudel* with a subsequent high vacuum distillation is favorable in contrast to the widely used simultaneous extraction/distillation according to *Likens-Nickerson.*

2.4.1.1 Simultaneous Extraction/Distillation According to *Likens-Nickerson*

As mentioned above, Likens and Nickerson designed an apparatus that combines extraction with simultaneous distillation (simultaneous distillation-extraction SDE). In this process, shown in **Figure 9**, low-boiling solvents are usually used to make subsequent concentration of the aroma substances easier. Therefore, this process is carried out at normal pressure or slightly reduced pressure. The sample is suspended in an aqueous solution and heated up in a flask above the boiling point (1). A second flask (2) containing the solvent (e.g. pentane) is heated as well above the solvents boiling point. Due to the diffusion of rising vapors, flavor compounds carried by the water vapor are assimilated in the reaction area by the solvent vapor. Vapors condense in the condensation area (3). Water phase and solvent separate in the condensate separator (4) before they return into their flasks. Within 0.5 to 2 hours, the solvent phase is enriched with flavor compounds.

Kalio [80] successfully isolated 69 aroma compounds from the press juice of arctic bramble (raspberry) by steam distillation. In Literature this technique is characterized by positive attributes like short processing time, small amounts of the required solvent, and a relatively high flavor concentration in the extract. But it comes along with several disadvantages such as high thermal treatment of the food samples that can lead to reactions that produce unwanted metabolism products or change the aroma composition. Furthermore, distillation/extraction according to *Likens-Nickerson* only allows the transfer of compounds that are soluble in water. Hence, it may not document the important, water insoluble flavor components [81].

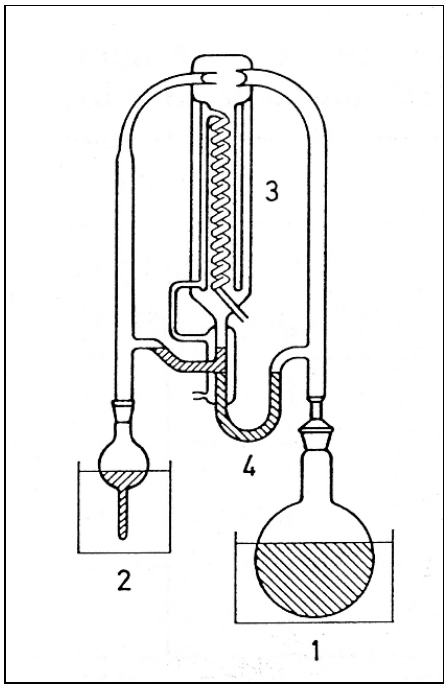


Figure 9: Apparatus according to *Likens* and *Nickerson* used for simultaneous extraction and distillation of volatile compounds.

Source: *Belitz,H.D.* et al. (2004) [24]

2.4.1.2 Liquid-Liquid Extraction According to *Kutscher-Steudel*

Extraction according to *Kutscher-Steudel* represents a very gentle liquid-liquid extraction (LLE) method since it requires low temperatures, normally around the boiling point of the used solvent. It is therefore recommended for temperature sensitive analytes such as the above mentioned metabolism products. Additionally, this method includes a much broader spectrum of flavor compounds due to their solubility in the solvent. The extraction process itself is very time consuming, requiring a period of about 20 hours followed by an independent distillation step. The latter might be a reason that only a few studies used this technique for flavor isolation. *Pyysalo* et al. [82] successfully examined the flavor volatiles of wild strawberries by using a modified *Kutscher-Steudel* extractor. *Dregus* et al. [83] analyzed alcohols and acids in rhubarb stalks, comparing it with simultaneous distillation-extraction (SDE) and vacuum headspace (VHS). They found that LLD and VHS give good comparable results while heat treatment during SDE affected the results negatively by higher amounts of heat induced degradation products.

The basic principle of a *Kutscher-Steudel* extraction apparatus is depicted in **Figure 10.**

An appropriate amount of sample material dissolved in water is given into the extractor flask (1). The used solvent in a separate flask (2) is heated gently above its boiling

point. The accruing vapor condenses in the cooler and flows through the fritte into the water-sample suspension (3). By taking advantage of their differences in density, the solvent goes up through the water phase, which contains the dissolved flavor compounds of the sample, and thereby assimilates them. Solvent enriched with flavor compounds runs back into the flask (2) and the cycle starts again. Finally, the extract is distilled by solvent assisted flavor evaporation (2.4.1.3).

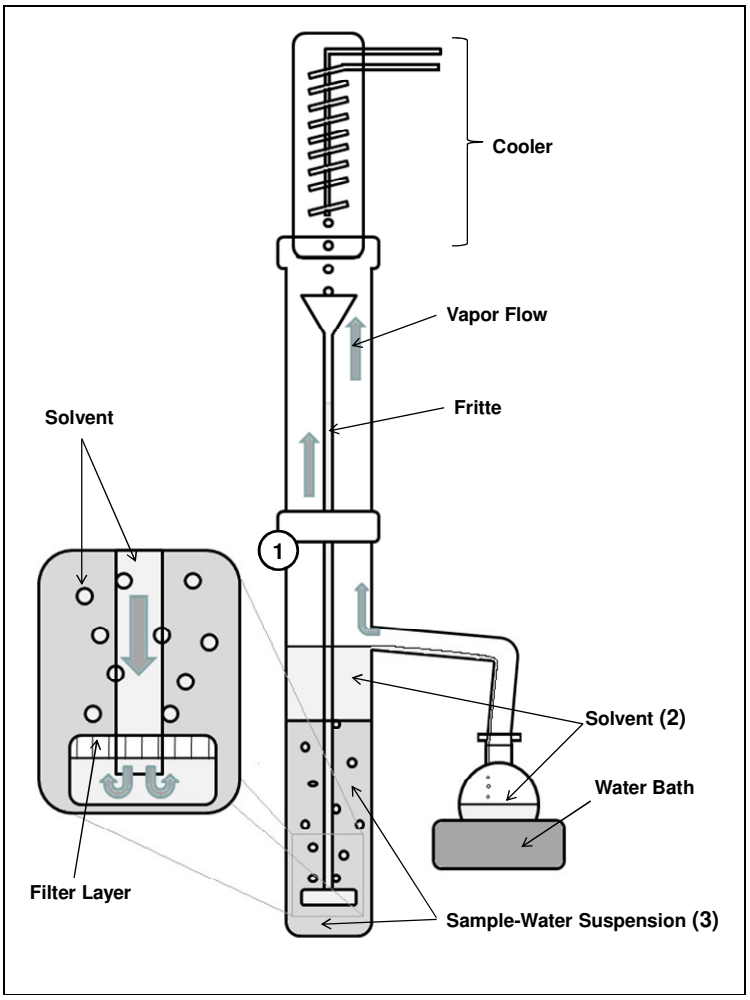


Figure 10: Liquid-liquid extractor according to *Kutscher* and *Steudel*.

2.4.1.3 Solvent Assisted Flavor Evaporation (SAFE)

Solvent assisted flavor evaporation (SAFE) is a relatively new and one of the most versatile techniques for the direct isolation of aroma compounds from complex food matrices. It was developed in 1999 by *W. Engel* et al. [84] as a fast and careful method for the isolation of volatiles from solvent extracts, aqueous food, aqueous food suspensions or even matrices with high oil content. The SAFE apparatus includes a compact distillation unit as shown in **Figure 11** in combination with a high vacuum pump. After appli-

cation of high vacuum (~5 mPa) the distillation procedure is started by dropping the liquid food or the extract from the funnel (1) into the distillation flask which is heated to 35-40°C in a water bath (2). The volatiles including the solvent vapor are transferred into the distillation head (3). The distillate is condensed by liquid nitrogen in the receiver (4). The drawer flask (5) protects the vacuum pump (reduced pressure 10^{-3} Pa). Solid foods require a previous extraction as described in chapter 2.4.1.1 and 2.4.1.2.

SAFE comes along with several advantages such as higher yields of volatiles compared with previously used high vacuum transfer techniques, higher yields of more polar flavor substances and odorants from fat-containing matrices. Furthermore, it allows the direct distillation of aqueous samples such as milk, beer, and orange juice, reliable quantification of polar and labile trace volatiles in complex matrices, compared with many other highly sophisticated modern methods, and recovers real authentic flavor extracts [24, 77, 85, 86].

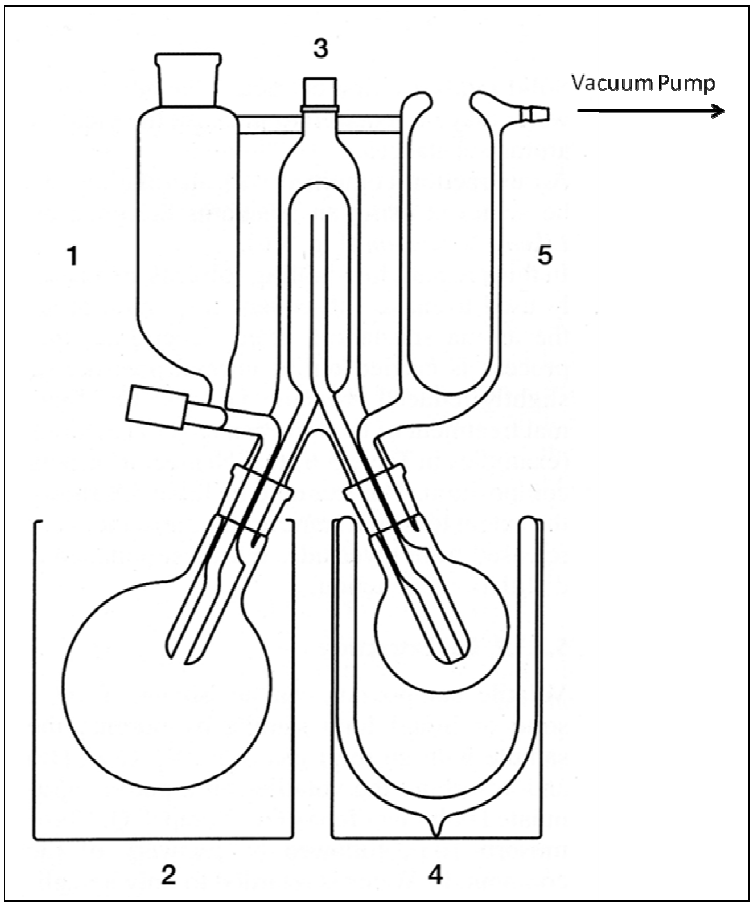


Figure 11: SAFE apparatus for the distillation of aroma substances from food (for explanations, see text. According to *Engel* et al. [24, 84])

Source: Belitz, H.D. et al. (2004) [24]

2.4.1.4 Headspace Sampling

Headspace collection is widely used because of its simplicity and the possibility of automation, allowing the analysis of a variety of samples. In general, the sample is sealed in a container, heated up to the desired temperature and left for a while to establish a equilibrium between volatiles bound to the sample matrix and those present in the vapor phase above. The possibility of artifact formation using this procedure is minimal because neither severe temperatures nor organic solvents are used. Also, losses during extraction and concentration are eliminated, although other losses may occur. However, this method can collect only the most volatile compounds. Often, the amounts of substance isolated in this process are so small that important aroma substances, which are present in food in very low concentration, give no detector signal. The complete picture of the flavor may be lacking, making a determination of important compounds possible only by sniffing the carrier gas stream (olfactometry) [24].

Equipment adjusted to the requirements, given by the sample is very important. *Jennings* and *Filsoof* [87] examined the influence of different gases and polymers on purging and adsorbing volatiles, showing major differences depending on the used materials. A comparison of some aroma compound isolation methods including the accosted headspace analysis showed that the results obtained by distillation-extraction procedure still give the best representation of the composition of the starting solution. However, the formation of artifacts is critical and the preparation time consuming [24].

The headspace analysis can be subdivided in two classes, the static and the dynamic procedure. Both show several advantages.

2.4.1.4.1 Static and Dynamic Headspace Analysis

By using static headspace, a given volume of the mentioned headspace is injected into a gas chromatograph equipped with a suitable separation column. Since the water content and an excessively large volume of the sample substantially reduce the separation efficiency of the following gas chromatography, only the major volatile compounds are indicated by the detector. However, the static headspace analysis makes an important contribution when the positions of the aroma substances in the chromatogram are determined by simultaneous olfactometry.

In the dynamic procedure, also called “purge and trap”, more material is obtained. The headspace volatiles are flushed out and purged with an inert gas (e.g. N_2, CO_2, He), adsorbed and thus concentrated on a porous, granulated polymer, followed by recovery of the compounds. Desorption of volatiles is usually achieved stepwise in a temperature gradient. At low temperatures, the traces of water are removed by elution, while at ele-

vated temperatures, volatiles are released and flushed out by a carrier gas into a cold trap, usually connected to a gas chromatograph.

However, it is difficult to obtain a representative sample by this flushing procedure, a sample that would match the original headspace composition [24, 88, 89].

2.4.2 Flavor Identification and Quantification

After isolating and concentrating the flavor compounds from the sample, identification and quantification is mostly carried out by a combined chromatography – mass spectrometry.

2.4.2.1 Chromatography

Chromatography is the collective term for a family of techniques for the separation of mixtures. In 1900, the botanist *Michael Semjenowitsch Tswett* was the first to separate several kinds of chlorophyll and xantophyll in a glass column packed with calcium carbonate and gave it its name which comes from the Greek *chroma*, color and *grafein*, to write.

Over the years, researchers found that the principles underlying *Tswett*'s chromatography could be applied in many different ways, giving rise to several varieties of chromatography which have all common properties. They all contain the necessary analyte which is located in the mobile phase, moving in a definite direction, and a stationary phase. Different chromatographic techniques can be classified by their basic attributes.

One of them is the bed shape which subdivides chromatography in two major groups.

On the one hand there's the column chromatography which contains a stationary bed within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column).

The planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a chromatography paper, serving as such or impregnated by a substance as the stationary bed, a so called paper chromatography, or a layer of solid particles spread on a support such as a glass plate, a so called thin layer chromatography.

A further way of differentiation is the physical state of the mobile phase which leads to three main techniques.

One of the most common is the gas chromatography (GC), also known as gas-liquid chromatography (GLC), a separation process in which the mobile phase is a carrier gas. It is always carried out in a column which can be packed as well as be capillary. Since

GC is extensively used in chemistry research and represents the used analytic method in this thesis, it will be described more preciously in chapter 2.4.2.2.

The liquid chromatography (LC) on the other hand uses a liquid as the mobile phase. It can be carried out either in a column or on a plane. The most common LC is the high performance liquid chromatography (HPLC). At this, the sample is forced through a packed column by a liquid at high pressure. HPLC is historically divided into two sub-classes, based on the polarity of the mobile and the stationary phase. A technique in which the stationary phase is more polar than the mobile phase is called normal phase liquid chromatography (NPLC) while the opposite is called reversed phase liquid chromatography (RPLC).

2.4.2.2 Gas Chromatography (GC)

As mentioned above, GC is a type of chromatography which involves a sample being vaporized and injected onto the head of the chromatographic column. A principle set-up is shown in **Figure 12**. The sample is transported through the column by the flow of a carrier gas (mobile phase), usually an inert gas such as helium or an unreactive gas such as nitrogen. The column itself contains a liquid stationary phase, a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing.

Two types of columns are used in GCs.

Packed columns are 1.5-10 m in length and have an internal diameter of 2-4 mm. The tubing is usually made of stainless steel or glass and contains a packing of finely divided, inert, solid support material that is coated with a liquid or solid stationary phase.

Capillary columns have a very small internal diameter, on the order of a few tenth of millimeters, and length between 25-60m are common. The inner walls are coated with active materials. Most are made of fused-silica with a polyimide outer coating. Those columns are flexible which makes it possible to wound a very long column into a small coil.

The nature of the coating material (stationary phase) determines what type of materials will be most strongly adsorbed. Thus numerous columns are available that are designed to separate specific types of compounds, for instance depending on their polarity.

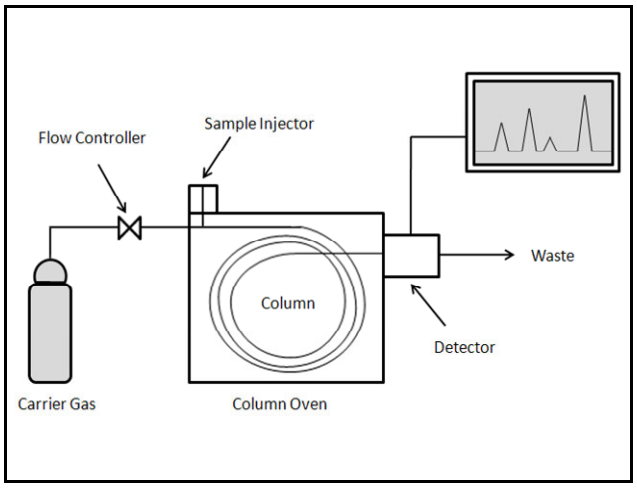


Figure 12: Principle set-up of a gas chromatograph.

Today, the injection is mostly carried out by an autosampler since automatic insertion provides better reproducibility and is less time consuming. As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by temperature and adsorption of the analyte molecules either onto the column wall or onto packing material in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. This can be influenced by choosing an adequate coated column depending on the attributes of the analyzed sample.

The rate at which a sample passes through the column is logarithmically influenced by the temperature of the column. Therefore the column in a GC is contained in an oven, the temperature of which is precisely controlled electronically. The higher the temperature, the faster the sample moves through the column. However, the faster the mobile phase move through the column, the less the analytes interact with the stationary phase, and the less they are separated. In general, the column temperature is selected to compromise between the length of the analysis and the level of separation. There are two ways of heat control. The isothermal method holds the column at the same temperature for the entire analysis whereas a so called temperature program increases the column temperature over the time which allows the analytes that elute early in the analysis to separate adequately, while it takes a shortening of time for late-eluting analytes to pass through the column. The individual time each molecule needs to pass the column is called retention time (rt).

When leaving the column, the separated molecules enter the detector. The detector is a sensing device used to monitor the concentration profiles of solutes eluted from the gas chromatography column. There are several detectors and each of them will give different types of selectivity. The most common are the flame ionization detector (FID) and

the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas, FIDs are sensitive primarily to hydrocarbons but can't detect water. Both are quite robust. Other detectors are flame photometric detector (FPD), mass selective detector (MSD), photo ionization detector (PID) and other. In most modern applications however the GC is connected to a mass spectrometer (MS) that is capable of identifying the analytes represented by the peaks. Since most of the flavor analysis described in literature as well as the analysis part of this thesis is carried out by using a combined GC-MS apparatus, this method will be described more detailed in chapter 2.4.2.4. Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis). This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. The retention time can be used to identify analytes if the method conditions are constant.

Since the area under a peak is proportional to the amount of analyte present, a quantitative analysis becomes possible. By calculating the area of the peak using the mathematical function of integration, the concentration of an analyte in the original sample can be determined. The concentration is mostly calculated by determining the relative response factor of an analyte. The relative response factor is the expected ratio of an analyte to an internal or external standard (a chemical added to the sample at a constant concentration, with a distinct retention time to the analyte) and is calculated by finding the response of a known analyte and a constant amount of the standard [24, 90].

2.4.2.3 Mass Spectrometry (MS)

Mass Spectrometry is an analytical technique that measures the mass-to-charge ratio (Q/m) of charged particles. When presenting data, it is common to use the dimensionless m/z, where z is the number of elementary charges (e) on the ion (z=Q/e).

Early devices were called mass spectrographs since they recorded a *spectr*-um on a phot-*ograph*-ic plate. In 1886, *Eugen Goldstein* was the first to observe "rays" that travelled through the channels of a perforated cathode in a low pressure gas discharge and moved toward the anode, in the opposite direction to the negatively charged cathode rays. In 1899, *Wilhelm Wien* constructed a device that separated the positive rays according to their charge-to-mass ratio via parallel electric and magnetic fields. In the course of time mass spectrometry was developed further what makes it today a widely used technique in the fields of analytical chemistry, pharmacokinetics, protein characterization and isotope dating and tracking.

All mass spectrometer consists of three basic parts as shown in **Figure 13**, an ion source, a mass analyzer, and a detector system.

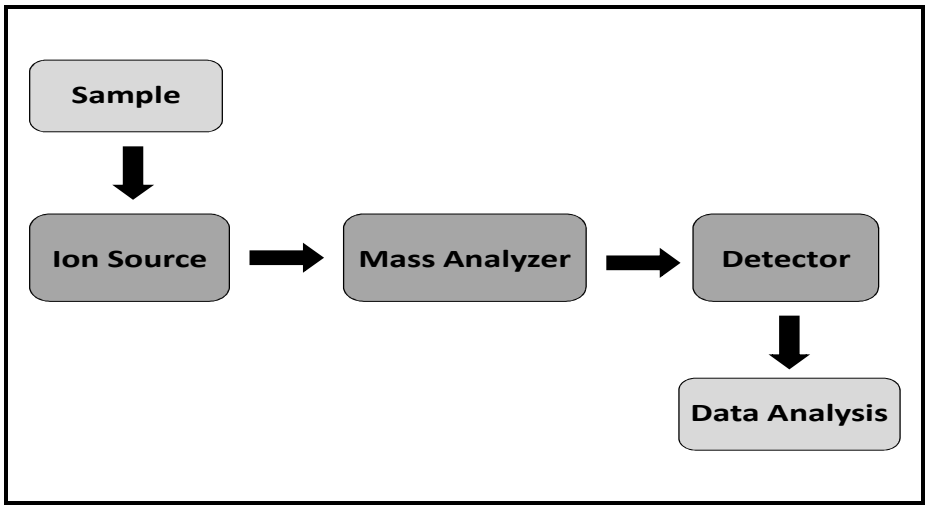


Figure 13: Basic parts of a mass spectrometer.

The ion source is the part of the MS that ionizes the analyte. Historically, there are different methods in ionization but only a few are dominating. The most common is the electron ionization (EI) in combination with gas chromatography for gases and vaporizable liquids and solids. Thereby, electrons with an energy of 5-200eV (most often 70eV) are generated and collide with the analyte molecule. The energy is transmitted on the molecule and primarily positive ions are generated. Those ions are mostly instable and brake completely or partly into smaller charged mass fragments. At this, every molecule breaks in a predictable pattern of ions.

The electrospray ionization (ESI), due to *John Fenn*, is applicable for larger molecules such as proteins. A chemical solution of charged or polar substances is sprayed, ionized, and the so created droplets dried so that ions of the analyte remain. Even by using a pulsed laser light, the analyte can be evaporated from a solid. This method is called matrix-assisted laser desorption/ionization (MALDI), due to *K. Tanaka* and separately, *M. Karas* and *F. Hillenkamp*. Thereby the analyte is mixed and co-crystallized in a disproportionately large amount of matrix material. The matrix material has the characteristic to absorb energy at the wave length of the used laser light. When the laser hits the matrix, the now positive charged analyte is set free. Further methods are chemical ionization (CI), field desorption (FD) and atmospheric pressure chemical ionization (APCI).

The mass analyzer separates ions by their masses or more precise, by their mass-to-charge ratio (m/q). All mass analyzers are based on dynamics of charged particles in electric and magnetic fields in vacuum where the *Lorentz force law* and the *Newton's second law of motion* applies.

There are many types of mass analyzer, using both static or dynamic fields, and magnetic or electric fields. Each of them has it strengths and weaknesses and they often differ substantially in their resolution power.

The resolution power is defined by the minimal mass difference *dm* of two ions to be separated from each other. The resolution of a mass analyzer is given by the unit *Th* (Thomson) and is different to the resolution power *R* which is given most often. The resolution power is the ratio of a mass to the mass difference of the closest separable mass (*R=m/dm)*. For example, while obtaining a resolution power of 4000, peaks of 4000 Th and 4001 Th could be separated as well as peaks of 2000 Th and 2000.5 Th, because 2000/(2000.5-2000)=4000.

Since it is used in this study, the sector field mass analyzer should be explained more detailed. It uses an electric and/or magnetic field to affect the path and/or velocity of the charged particles. The radius of the circular path within the fields depends on the energy (in the electric field) and the impulse (in the magnetic field) of the ions. Knowing the charge, the energy, and the impulse makes it possible to determine the mass. Sector field mass analyzer can be designed to image ions of a slightly different speed (velocity focusing) or slightly different directions (direction focusing) at one point of the detector. Most modern sector instruments do both by combining a magnetic and an electrostatic sector field and are called double focusing mass analyzer (**Figure 14**). The focusing is necessary to reach an acceptable intensity of the signal at resolution powers up to R=100.000. That makes them the most precise but also the most expensive mass analyzer.

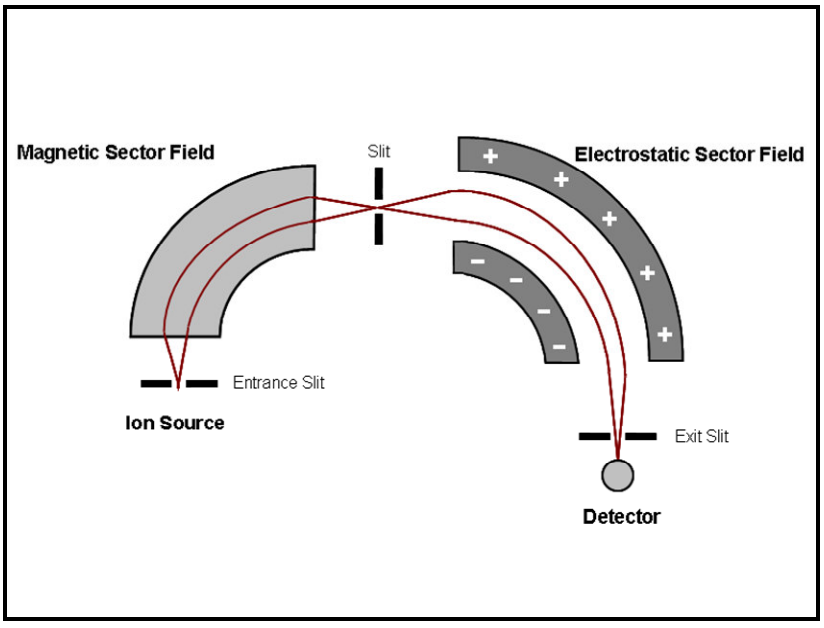


Figure 14: Design and function principle of a double focusing mass analyzer.

Other common mass analyzers are for instance time-of-flight (TOF) or quadrupole analyzer. The former is taking advantage of an identical energy of all ions, entering the analyzer. Lighter ions reach the detector earlier since they are faster than heavier ones.

The resolution power of modern TOF mass spectrometers is about R=15.000. Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize ions passing through a radio frequency quadrupole field. They act as mass selective filters what means that only stable ions of a defined mass (or rather mass-to-charge ratio) can pass through the alternating field inside four parallel electrodes, regulated by adjusting the frequency ω or voltage *U*, *V*.

Further mentionable mass spectrometers are tandem mass spectrometers (MS/MS) and several types of ion trap spectrometers such as quadrupole ion trap, linear trap, FT-ICR-MS and orbitrap.

A mass spectrometer is typically utilized in one of two ways. When collecting data in the full scan mode, a target range of mass fragments is determined. The used range is largely dictated by the expected fragments in the sample. However, a MS should not be set to look for mass fragments too low or else one may detect interferences such as air, found as m/z 28 due to nitrogen or m/z 44 due to carbon dioxide. A typical range of mass fragments to monitor would be m/z 50 to m/z 400. A range too broad also decreases the sensitivity of the instrument due to performing less scans per second, each detecting a wide range of mass fragments.

The second mode is the selective ion monitoring (SIM). At this, only a certain range of ion fragments are entered into the instrument method and only those are detected by the MS. The advantage lies with this limited number of fragments the instrument is looking for during each scan. Furthermore, more scans take place each second which increases the accuracy of the results [90, 91].

2.4.2.4 Gas Chromatography–Mass Spectrometry (GC-MS)

GC/MS is a method that combines the features of gas-liquid chromatography (GC) and mass spectrometry (MS) to identify different substances within a test sample. It is composed of two major building blocks, the gas chromatograph (2.4.2.2) and the mass spectrometer (2.4.2.3). While the GC uses differences in the chemical properties between different molecules in a mixture to separate the molecules as the sample travels through the length of the column, the MS downstream captures, ionizes, accelerates, deflects and detects the molecules, or rather their ionized fragments, separately, using their mass-to-charge ratio. A simplified set-up of a modern GC/MS is shown in **Figure 15**.

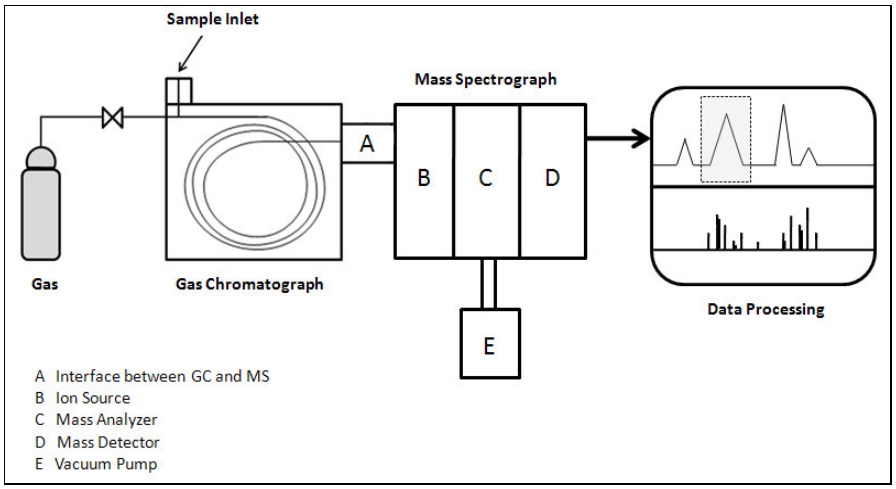


Figure 15: Simplified set-up of gas chromatograph / mass spectrograph unit.

In December 1955, *Roland S. Gohlke* and *Fred W. McLafferty* were the first to couple a gas chromatograph to a time-of-flight mass spectrometer [92]. This unique GS/MS instrument was bulky, fragile, and originally limited to laboratory settings. It generated mass spectra at a 10-KHz rate for display on an oscilloscope. Eluted gas chromatographic components, such as methanol, aceton, benzene, toluene, and carbon tetrachloride could be visually identified immediately from the oscilloscope display. Many years of further research and the development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvement in the amount of time it takes to analyze a sample making on-line GC/MS the uniquely valuable analytical tool that it is today.

The main advantage in using GC and MS together as one analytical tool is a much higher degree of substance identification than each of them can provide when used separately. It is hardly possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The MS process normally requires a very pure sample and sometimes two different molecules can have a similar pattern of ionized fragments. The GC, using a traditional detector, may detect multiple molecules of the same retention time which results in two or more molecules to co-elute. Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC/MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.

Today, fast and accurate results have led to a widespread adoption of MS in a number of fields such as in forensic science, security, medicine, astrochemistry and food, beverage and perfume analyses [90, 91, 93, 94].

2.4.3 Quantification

2.4.3.1 Internal Standards

An internal standard in analytical chemistry is a chemical substance that is added in a constant amount to samples. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards.

Quantification of the eluted species is relatively straightforward in determinations involving a single analyte but is complicated in more usual cases by the large number and chemical diversity of volatile compounds. Selection of appropriate internal standard(s) is then difficult, although *Molteberg* et al. [95] demonstrated that toluene is capable to quantify a broad range of volatiles.

The internal standard should not be any natural compound of the sample to avoid variances due to addition but should be a compound that matches as closely, but not completely, the chemical species of interest in the samples, as the effects of sample preparation should, relative to the amount of each species, be the same for the signal from the internal standard as for the analytes signal(s). Since sample preparation affects every single chemical compound in a different way, the use of conventional standards can never lead to an accurate quantification.

2.4.3.2 Stable Isotope Dilution Analysis

Stable isotope dilution analysis is a relatively new and powerful technique that has allowed accurate quantification of several compounds in popcorn [96], toasted bread [97, 98, 99] and sourdough fermentation [100].

In this procedure, isotopomeres are used as internal standards, and many of these must be synthesized. In mass spectrometry, the non-radiating isotopes ^{2}H (deuterium, D) and ^{13}C are preferably employed and thus, a rich methodology to incorporate isotopic labels has been developed [90].

The procedure provides highly accurate measurements since unavoidable losses of analyte during isolation are ideally compensated, although the timing of addition of the spike must be considered. *Zehentbauer* and *Grosch* [98, 99] quantified several volatiles in white bread crust using stable isotope dilution analysis and spiked the sample with the relevant isotopomers after grinding it in a frozen state.

The chemical state of the spike vis-à-vis the native analytes is a potential concern as is adequate mixing of the spike and the sample. However, these are relatively minor considerations, and today the stable isotope dilution analysis represents state of the art in quantification [66, 101].

2.5 Color and Color Measurement

Color is an important factor in determining food preference. People learn to expect certain colors in their food and any variation from the expected color will lower the preference. Brown colors, particularly in cereals, are a key factor in consumer acceptance [102, **Error! Bookmark not defined.**].

Extruded cereals show a typically golden to light brown color that is well accepted by the consumer. Looking at todays popular whole grain cereals, part of this color is due to natural dyeing pigments, which are xantophyll (lutein type), carotene, flavones, and cryptoxanthine, located in the outer layers of the kernel.

A second and more important account for brown coloration is the Maillard reaction between reducing carbohydrates and compounds bearing an amino group, occurring during thermal processing of food. Depending on their molecular weight, the colored components affecting this nonenzymatic browning may be divided into two classes, namely, the lower molecular weight colored compounds, consisting of up to four linked rings with molecular weights <1.000 Da, and the melanoidins, which are assumed to be water-soluble, high molecular weight colored compounds with masses up to 100.000 Da. Especially low molecular weight compounds are often potent flavor compounds [103, 104, 105, 106, 107].

If color can be expressed in concentration units, it might be possible to relate color directly to the rates of formation what would make color measurement a helpful tool in process controlling. Until now, only a few attempts are reported, trying to show a correlation between color development and flavor characteristics. *Farouk* et al. [62] examined the possibility to obtain products of satisfactory aroma even under conventional extrusion conditions by increasing the rate of browning reactions during extrusion cooking.

The intensity of browning can be measured in different ways. The main methods involve visual comparison with standard references, chemical pigment extraction, and light reflectance measurements. In recent years, near-infrared reflectance (NIR) and near-infrared transmittance (NIT) and, more recently, UV-vis spectrophotometers have been used to determine color as a parameter useful to assess food quality. However, in food industries, the most popular color measurement is based on the refractometer color method. Its reproducibility was studied by *Ramirez-Jimenez* et al. [108] on commercial snack bread. The success of this automatic technique can be found in the rapidity and safety of the procedure in addition to the good correlation found between the conventional chemical extraction and the reflectance measurements [109, 110, 111].

The refractometer color method is using the L*a*b* color-space system, developed by the Commission Internationale d'Eclairage (CIE) [112]. It describes all the colors visi-

ble to the human eye within a three coordinates diagram. In the CIE L*a*b*, L* represents the lightness (L*=100 yields black, L*=0 indicates white) while a* represents red and green (negative values = green, positive values = red) and b* yellow and blue (negative values = blue, positive values = yellow) [108].

Differentiation of red, yellow, green and blue in the L*a*b* color-space system are of high interest since it is well-known that orange or red pigments are formed in early stages of nonenzymatic browning, representing compounds of low molecular weight while green and blue pigments are characteristic of high molecular weight melanoidins. It has to be taken into account that extrusion cooking represents a short-time process; favoring the formation of low molecular weight colored compounds in early stages of the Maillard reaction and the complex interaction of different process parameters such as retention time, temperature, and additional flavor precursors can influence color and flavor generation. Hence, it remains unclear if color measurement may help to evaluate the influence of process parameters on flavor development [62].

3 Materials and Methods

3.1 Raw materials

Wheat starch (Manildra Milling Corp., Shawnee Mission, KA, USA) was the base of all generated directly expanded extrudates. “Spray Process Grade A Nonfat Dry Milk” (Dairy America Inc., Fresno, CA, USA), “Whey Protein Isolate” (Davisco Inc., Le Sueur, MN, USA), and the amino acids cysteine, proline and leucine and valine (>99% p.a., Sigma-Aldrich, Buchs, Switzerland) were used as additional amino sources. Compositions of the protein additives are shown in **Table 4**.

Table 4: Proximate composition of protein containing additives used

Components	Milk Powder	Whey Protein isolated
Moisture [%]	3.9	4.2
Protein [%]	33.4	94.0
Fat [%]	0.8	0.1
Minerals [%]	7.8	1.7
Lactose [%]	54.1	0.0

Wheat starch was enriched with milk powder or whey protein to achieve a total protein content of 0% to 3%. Both were mixed to the desired ratio in a ploughshare mixer for 5 minutes to ensure the homogeneity of the feeding material before extrusion.

The amino acids were dissolved in distilled water at room temperature and added to the extrusion process by an electromagnetic flow measuring system “Promag 50” (Endress+Hauser, Greenwood, IN, USA) to achieve final concentrations in the analyzed sample material of 250 and 1.000 ppm.

Salt (Cargill Inc., Minneapolis, MN, USA) and sugar were dissolved in water, creating slurries which were fed into the extruder by an electromagnetic flow measuring system “Promag 50” (Endress+Hauser, Greenwood, IN, USA) to achieve a target concentration in the product of 0.75% salt and 0% to 8% sugar.

Two different sugars, glucose syrup “Howell” (Cargill Inc., Eddyville, IO, USA) and glucose (Tate & Lyle Ingredients Americas Inc., Dacature, IL, USA), were used separately to compare their influence on flavor formation. The glucose syrup used is charac-

terized by a dextrose equivalent (DE) of 63 containing approximately 37.5% glucose, 34.2% maltose, 16.1% higher sugars and 12.2% dextrins at a solid content of 82%.

3.2 Sample Generation

3.2.1 Experimental Design

Extrusion trials were made using factorial designs. Fractionized factorial trials were realized by the Central-Composite-Design. A statistical evaluation of the achieved results was made by ANOVA (Analysis of Variance) and the software "DesignExpert®" (Version 6.0.5., Stat-Ease, Minneapolis, USA).

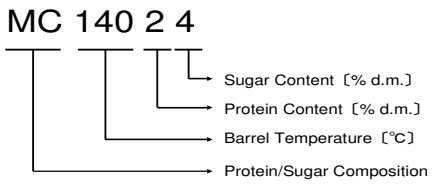
Changeable parameters were barrel temperature as well as protein and sugar content, each varied over 3 levels as shown in **Table 5**.

Table 5: Designs of the extrusion cooking experiments

Parameter	**Level**		
	-1	**0**	**+1**
Barrel Temperature [°C]	140	160	180
Protein [% DS]	1,3	2,0	2,6
Sugar [% DS]	2	4	6

3 Designs were carried out, differing in the composition of glucose and glucose syrup as well as whey protein and milk protein. The compositions and their codings are shown in **Table 6**.

Table 6: Coding of the experimental trials

Composition Protein Source/Sugar	Coding	Total Coding
Milk Powder/Glucose syrup	MC	
Milk Powder/Glucose	MG	
Whey Protein/Glucose	WG	

3.2.2 Extrusion Process

The extrusion processing was carried out on a twin-shaft extruder "DNDL-44/28D" (Bühler AG, Uzwil, Switzerland) with seven barrel sections containing independently controlled heating (3) and cooling (1) zones. A "circulating liquid temperature control system" (MOKON, Buffalo, NY, USA) was used to heat the barrels in a range from 140 to 180°C. The gelatinized starch was extruded through a die plate containing 2 round holes of 2 mm diameter each. The screw consisted of conveying elements with different pitches only. Seven reverse elements where put in place in the last third of the screw to increase the input of mechanical energy. The technical data of the extruder are shown in **Table 7**. The general profiles of the barrel temperature and the mechanical energy input are depicted in **Figure 16**.

Barrel temperature, die temperature, die pressure, shaft torque, extruder power, specific mechanical energy input (SME), and feed rates were measured and controlled by an extruder control system "BCTB" (Allen Bradley, Milwaukee, WI, USA). Solid raw material was fed into the first barrel section, cooled to room temperature, by a K-Tron series "K10S" gravimetric feeding system (KTron Ltd., Niederlenz, Switzerland). The moisture content of the feed was controlled by injecting water at room temperature (23°C) into the extruder with an electromagnetic flow measuring system "Promag 50" (Endress+Hauser, Greenwood, IN, USA). The final mass flow rate has been kept constant at 80 kg/h. The screw speed was 350 rpm constantly. The expanded extrudate was pelletized by an adjustable three blade cutter at the face of the die. It was operated at 1.100 rpm constantly. Due to a constant screw speed, the residence time was expected to remain constant at around 30 seconds. Therefore, separate measurements have not been conducted within this thesis.

Table 7: Technical Data of the Twin-Screw-Extruder DNDL-44/28D

Feature	**Specification**
Throughput capacity	20 … 80 kg/h
Motor power at constant torque	18.9 kW
Working torque	190 Nm/shaft
Screw speed	60 … 450 min^{-1}
Screw diameter (D)	44 mm
Screw length (L)	28 x D

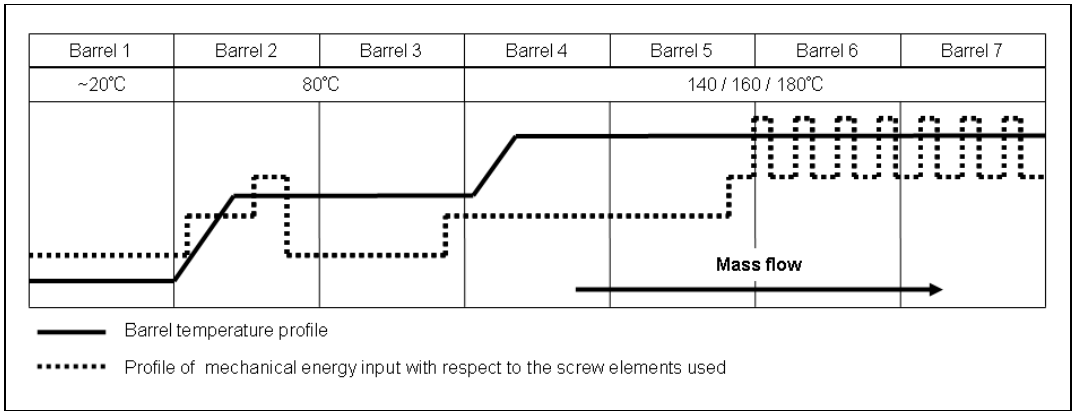


Figure 16: Barrel temperature profile and profile of the mechanical energy input in the extruder due to the heating of the barrel section and the use of different screw elements.

3.2.3 Drying Process

Drying was realized by using a fluid-bed cooler/dryer "OTW 50" (Bühler AG, Uzwil, Switzerland) coupled with a customized heat exchanger (Xchanger, Inc., Hopkins, MN, USA).

The pelletized extrudates were conveyed from the extruder to the dryer by an exhausting pipe, passing a cyclone which separated the extruded pellets from generated dust.

The drying time for each run was 2 minutes at a constant temperature of the heated air of 134°C. To guarantee the same drying time for each pellet in every single run, a defined amount of sample was collected in a basket that matched the inner dimension of the dryer before starting the fluid-bed drying process. The undried samples were collected when leaving the cyclone to avoid shrinkage that would have been occurred if the samples had been collected immediately after extrusion and cutting.

3.2.4 Sampling

After drying, 500g of each sample were collected in plastic polyamid-polyethene bags, cooled to room temperature, finely ground and stored at -20°C in glass jars until required for analysis

3.3 Isolation of Flavor Volatiles

3.3.1 Extraction according to *Kutscher-Steudel*

50 g of finely ground sample dissolved in 250 mL of distilled water was transferred into the Kutscher-Steudel extraction flask (shown in **Figure 10**) and filled up with distilled water to a total of 1L. The sample solution was covered with the needed amount of diethyl ether, used as the solvent, to reach the outlet of the flask. An additional 100 mL of diethyl ether was given into the 250 mL receiving flask which was heated in a water bath up to its boiling point. The evaporating solvent condensed in the condensation area, flowing through a fritte to the bottom of the extraction flask. Due to their density differences, the solvent rose through the sample solution accumulating with flavor components. The emerging solvent excess flowed back into the receiving flask where the cycle started again. The diethyl ether got enriched with flavor components over a period of 20 hours.

3.3.2 Solvent Assisted Flavor Evaporation (SAFE Method)

Extract obtained by extraction according to Kutscher-Steudel was added to the dropping funnel of the SAFE apparatus "BAENG" (Glasbläserei Bahr, Manching, Germany) (2.4.1.3), which was heated to 40°C in a water bath. The receiving flask for the distillate and the safety cooling trap of the instrument were cooled with liquid nitrogen. The SAFE apparatus was connected to a high vacuum pump "Trivac D16B" (Leybold-Heraeus, Hanau, Germany) and then the extract in the dropping funnel was added in small aliquots to the distillation flask over a period of 30 min.

The collected frozen distillate was thawed at room temperature. The extract was dried over anhydrous sodium sulphate, >99% p.a. (Roth GmbH, Karlsruhe, Germany) and concentrated to about 1.0 ml using a Vigreux column and water bath at 40-50°C. The extract was transferred into a vial. The distillation flask was washed with a minimum of diethyl ether, and the washing was added to the vial. The solvents were carefully removed using a purified nitrogen stream until the total volume was reduced to approximately 100 µl. The extract was then analyzed by GC/MS (3.4.1).

3.3.3 Purge and Trap Headspace Sampling

Purge and Trap dynamic headspace sampling was realized by using a "Tekmar 3000 Concentrator w/2016 ALS" (Teledyne Tekmar, Mason, OH, USA) equipped with a Tenex trap. 40 µl of 4-heptanone as an internal standard were added to 3 g of finely ground sample, sealed in a 40 ml vial and put in the auto sampler. The purge and trap parameters are given in **Table 8**.

Table 8: Purge and Trap instrument conditions

Parameter	Setting
Purge ready temperature	30°C
Purge temperature	30°C
Turbocooler temperature	100°C
Sample heater	ON
Pre purge time	2 min
Pre heat time	3 min
Purge vessel heater	60 °C
Purge time	20 min
Dry purge time	4 min
GC start	Des End
Cryofocuser	ON
GC cycle time	49 min
Cryo standby temperature	190
Cryofocus temperature	-50°C with liquid nitrogen
Injection time	2 min
Cryo injection temperature	190°C
Desorption pre heat temperature	180°C
Desorption temperature	200°C
Desorption	6 min
Sample drain	OFF
Bake time	20 min
Bake temperature	210°C
BGB	ON
Delay	2
Transfer line temperature	180°C
Valve temperature	150°C
20XX line temperature	130°C
20XX valve temperature	130°C
MCS temperature	100°C
Vial size	40 ml

3.4 Identification and Quantification of Flavor Volatiles

3.4.1 Gas Chromatography / Mass Spectrometry (GC/MS)

When headspace sampling was carried out, a quadrupole GC/MS was used while a double focusing GC/MS was used when flavor isolation was realized by extraction/distillation.

3.4.1.1 Quadrupole GC/MS

The system consisted of a Gas Chromatograph "Agilent 5890 series-II GC" (Agilent Technologies, Santa Clara, CA, USA) with a splitless injector and a linear temperature program (injection temperature 190°C, detector temperature 240°C) coupled with a quadrupole mass spectrometer "HP 5989 MS-ENGINE" (Hewlett-Packard Co., Palo Alto, CA, USA). A 30 m long and 0.25 mm i.d. fused silica capillary column "Rtx® Volatile" (RESTEK, Bellefonte, PA, USA) was used. Helium carrier gas was used at a column head pressure of 97 kPa (14 psi). The oven temperature was programmed from 40°C (5 min isothermal) to 240°C (held for 10 min at final temperature) at 6°C/min.

Important parameters of the MS are given in **Table 9**. The GC/MS was equipped with an electronic data processing system "Wiley library 7N" (Wiley, Hoboken, NJ, USA).

Table 9: Parameters of the quadrupole mass spectrometer "HP 5989 MS-ENGINE"

ionization energy	70 eV (electron impact ionization)
scan range	40 to 450 Da
emission	n.s.
resolution	4 000

3.4.1.2 Sector field GC/MS

The system consisted of a Gas Chromatograph "HP GC 5890 Series 2" (Hewlett-Packard Co., Palo Alto, CA, USA) with a split-splitless injector and a linear temperature program (injection temperature 230°C, detector temperature 280°C) coupled with a double focusing sector field mass spectrometer "Model 8230" (Varian-MAT, Bremen, Germany). A 60 m long and 0.32 mm i.d. DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA, USA) was used. Helium carrier gas was used at a column head pressure of 150 kPa (22 psi). The oven temperature was programmed from 70°C (4 min isothermal) to 280°C (held for 30 min at final temperature) at 2°C/min.

Important parameters of the MS are given in **Table 10**. The GC/MS was equipped with an electronic data processing system "Maspec II[32]" (Mascom, Bremen, Germany).

Table 10: Parameters of the double focusing sector field mass spectrometer "Model 8230"

ionization energy	70 eV (electron impact ionization)
scan range	35 to 600 Da
emission	1 mA
resolution	100 000

3.4.2 Quantification

Quantification via stable isotope dilution analysis was carried out by the use of deuterium labeled 2,4-decadienal and furanmethanol and by carbon 13 labeled butyric acid. Additionally, trimethylpyrazine was added as an internal standard in unmodified state to quantify the amounts of other generated pyrazines and 1-octanol as a common internal standard, not naturally occurring in the original sample. The added amount of isotopic labeled standards and of trimethylpyrazine depended on the expected amount of the analogous analytes in the sample. The used standards and their amounts are listed in **Table 11**.

All standards were obtained from the chair for molecular analysis, Department of Biotechnology at the Technische Universität Berlin (Berlin Institute of Technology), Germany.

Table 11: Used internal standards and their amounts

Internal Standard	Concentration
1-Octanol	2 ppm
Trimethylpyrazine	1 ppm
D-2,4-Decadienal	0.2 ppm
^{13}C-Butyric Acid	2 ppm
D-Furanmethanol	2 ppm

3.5 Color Measurements

Color measurement was carried out with a tristimulus color analyzer “Minolta Chroma Meter CR-410” (Konica Minolta Sensing Americas, Inc., USA).

The method used is applicable to all non-enrobed puff cereal products and measures the color on the L*a*b* scale.

Approximately 100 g pre-ground sample material (room temperature) was ground for at least 20 seconds. After this second particle size reduction a powder was obtained, the particle size distribution of which was uniform enough to carry out reliable color measurements. Before measuring, the color meter was calibrated as described in the manual. The tip of the measuring head was pressed firmly onto the surface of the sample, which was filled into a 100 mL sample cup, by twisting the measurement head 3-4 times. A pulsed xenon arc lamp provided illumination on the sample surface and the light was reflected back to the instrument and measured. Three measurements were taken and the average results were displayed.

The procedure was repeated 3 times for each sample to achieve a maximum of statistic certainty.

4 Results and Discussion

The objective of this work was to examine and evaluate the influence of barrel temperature, protein and sugar content, and protein and sugar type on flavor development in extrusion cooked starchy raw material. The complex interactions within the process of extrusion are illustrated in **Figure 17**. It shows an adaptation of the systems analytical model developed by *Meuser* and *van Lengerich* for extrusion cooking of starch containing raw materials [113, 114]. It describes the influence of process parameters on system parameters and final product parameters as well as of system parameters on final product parameters. In contrast to former studies using the systems analytical model, the focus was primarily on how selected process parameters influence specific product parameters. The influences of process on system parameters as well as of system on product parameters were investigated but remained of secondary interest. The results showed a sufficient characterization of the relation between process and individual product parameters.

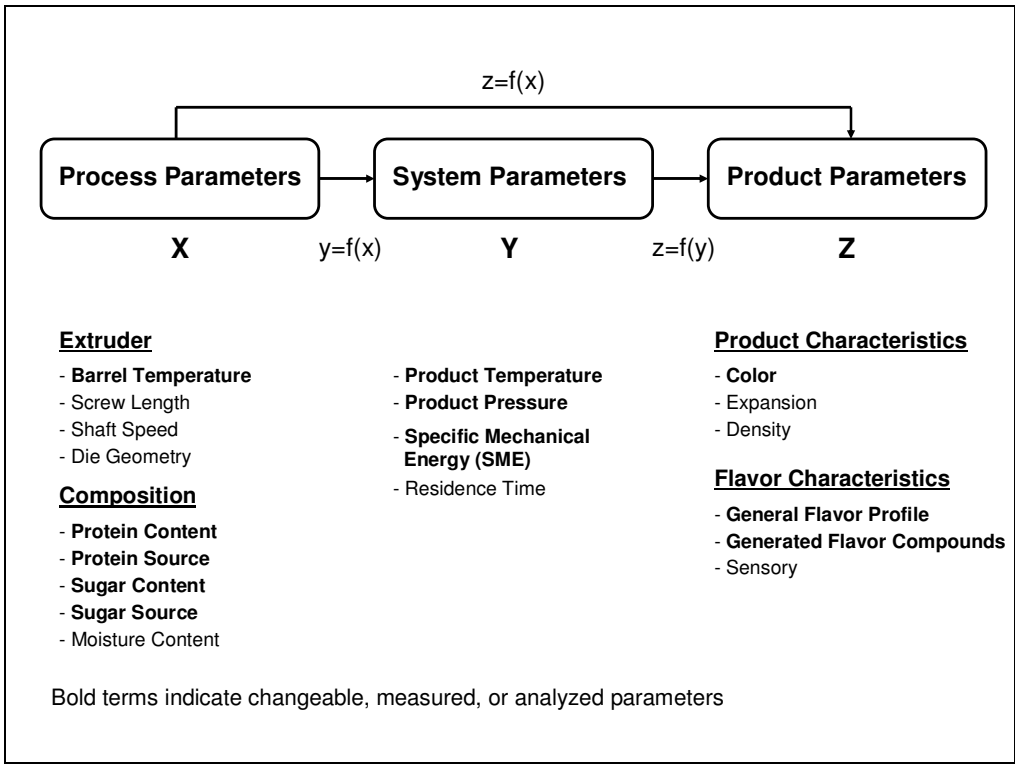


Figure 17: Systems analytical model for the extrusion cooking process

Aside from evaluating the influence of sugar, protein, and temperature, two different techniques of sample preparation for flavor analysis were carried out and compared regarding their specific advantages and disadvantages.

At last, color measurement was carried out to look for differences between the used sugars and proteins and to check on possible process controlling properties by color measurement.

4.1 Flavor Analysis

4.1.1 Comparison of Dynamic Headspace Sampling and Liquid/Liquid Extraction – SAFE Distillation

Flavor analysis was conducted by using different techniques of sample preparation. Dynamic headspace sampling (DHS) was compared to a combination of liquid/liquid extraction and SAFE-distillation (LLE-SAFE). Final analysis was made by gas chromatography/mass spectrometry (GC/MS).

The number of detected compounds depended on the chosen sensitivity of the evaluation software and the sample preparation technique. The total number of volatiles detected in samples analyzed by headspace-GC/MS ranged from 70 to 100 compounds within a retention time of 30 minutes. Using liquid-liquid extraction and SAFE distillation, the subsequent GC/MS analysis showed total amounts of 40 to 50 compounds within the same period of time. This is due to a high number of negligible, aroma inactive components, mostly alkanes, found by headspace analysis. A complete listing of compounds found by headspace sampling for all analyzed designs is enclosed in the appendix, **Table A 1** to **Table A 3**.

An exemplary comparison of the resulting gas chromatograms extracted from the extrudates is depicted in **Figure 18**. It shows differences in the detectable volatile compounds depending on the preparation technique and subsequent GC/MS.

Only a fraction of the total amount could definitely be named as aroma active components. A total of 28 flavor volatiles were identified. 21 have been found by headspace analysis and 23 by LLE-SAFE. A general overview on the detected aroma active compounds is given in **Table 12**.

By comparing the findings of both techniques, one main difference becomes visible. As already known and confirmed by several studies, more highly volatile compounds were detected by headspace-GC/MS while more less-volatiles have been found in samples prepared by extraction/distillation. Pyrazine and 2- and 3-methylbutanal were detected exclusively by headspace GC/MS. All three are important, high-volatile flavor compounds, responsible for desired caramel-like, roasted, and cereal flavor impressions.

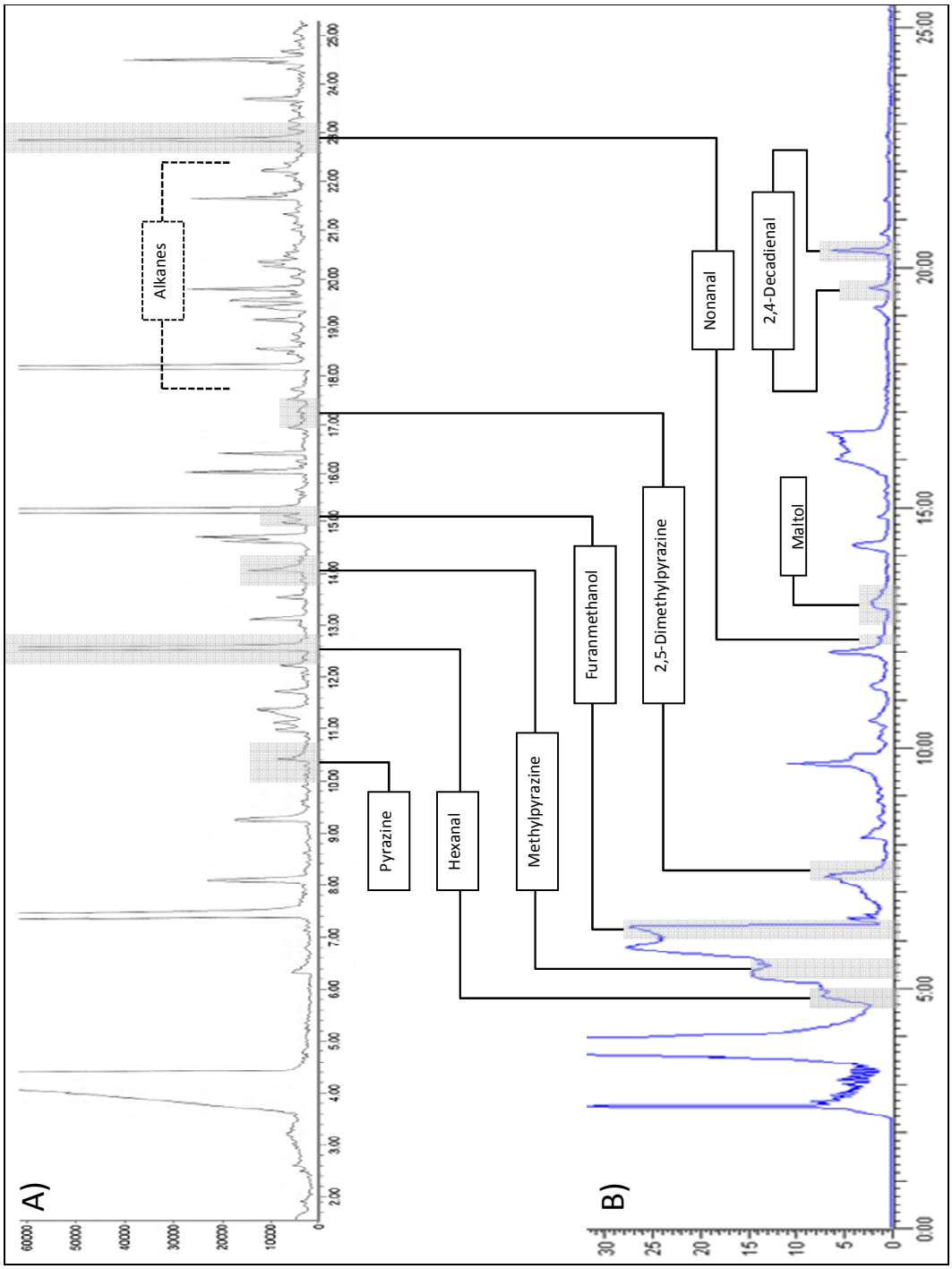


Figure 18: Chromatograms of extruded wheat starch, containing 6% whey protein and 4% glucose, processed at 160°C. A): headspace sampling with subsequent quadrupole GC/MS, B): liquid-liquid extraction/SAFE distillation with subsequent sector field GC/MS.

Table 12: Aroma active volatiles detected in extruded wheat starch samples containing different proteins and sugars, processed at 160°C, isolated by DHS and LLE-SAFE

Constituents	DHS			LLE-SAFE		
	M/C	M/G	W/G	M/C	M/G	W/G
Pyrazines						
pyrazine	x	x	x	n.d.	n.d.	n.d.
methylpyrazine	n.d.	x	x	x	x	x
2,5-dimethylpyrazine	n.d.	x	x	x	x	x
Aldehydes						
2-methylbutanal	x	x	x	n.d.	n.d.	n.d.
3-methylbutanal	x	x	x	n.d.	n.d.	n.d.
2-methylpentanal	x	x	x	n.d.	n.d.	n.d.
hexanal	x	x	x	x	x	x
furancarboxaldehyde	x	x	x	x	x	x
benzaldehyde	n.d.	n.d.	n.d.	x	x	x
2,4-decadienal	n.d.	n.d.	n.d.	x	x	x
heptanal	x	x	x	x	x	x
nonanal	x	x	x	x	x	x
Alcohols						
butanol	x	x	x	x	x	x
pentanol	x	x	x	x	x	x
furanmethanol	x	x	x	x	x	x
2,3-butanediol	n.d.	n.d	x	n.d.	n.d.	x
Acids						
acetic acid	x	x	x	n.d.	n.d.	n.d.
butyric acide	x	x	x	x	x	x
2 methyl butyric acid	n.d.	n.d.	n.d.	x	x	x
pentanoic acid	n.d.	n.d.	n.d.	x	x	x
hexanoic acid	n.d.	n.d.	n.d.	x	x	x
Ketones						
1-hydroxy-2-propanone	x	x	x	x	x	x
2,3-pentanedione	x	x	x	x	x	x
3-hydroxy-2-butanone	x	x	x	x	x	x
4 hydroxy-4-methyl-2-pentanone	x	x	x	x	x	x
Furanones / Pyranones						
dihydro-2-methyl-2(3H)-furanone	x	n.d.	n.d	x	x	x
maltol	n.d.	n.d.	n.d.	x	x	x
Furanes / Pyranes						
3,4 dihydropyran	n.d.	n.d.	n.d.	x	x	x
2-pentylfuran	x	x	x	x	x	x

x detected
n.d. not detected
M/C milk protein/glucose syrup
M/G milk protein/glucose
W/G whey protein/glucose

Various reasons are conceivable why those compounds have not been found by GC/MS and previous extraction/distillation. On the one hand, multiple preparation steps may have led to a loss of high-volatile compounds. On the other hand, the used separation column might have promoted the separation of less-volatile compounds at the expense of the high-volatiles.

However, different components were detected in samples prepared by LLE-SAFE that have not been found by headspace analysis. As an example, diverse acids, 2,4-decadienal, and maltol should be named. Additionally, vanillin was detected in several samples in trace amounts but should only be mentioned for the purpose of information. It represents a potent flavor compound that is not volatile enough to be detected by headspace analysis.

It was found that both analysis techniques come along with advantages and disadvantages. None is unambiguously preferable over the other.

On the one hand, headspace analysis offers a rapid analytical method for detecting volatile compounds. The use of an autosampler enables the analysis of up to 50 samples via headspace-GC/MS within 24 to 48 hours and makes headspace-GC/MS a versatile tool for industrial flavor analysis. However, less-volatile flavor compounds remain undetected, limiting an accurate analysis.

On the other hand, extraction/distillation is a very time consuming technique that required at least 24 h for preparation. Additionally, a maximum of 2 samples could be handled at the same time. One advantage lies within the detection of important less-volatile flavor compounds. Furthermore, the final analyte contained higher yields of volatile constituents due to a longer extraction time, resulting in an increased chance of detection. This is confirmed by findings from *Park* et al. [86]. Verification within this study remains complicated since quantification of the detected flavor compounds was made differently, depending on the preparation technique. 4-hepatanone was used as an internal standard during headspace analysis, while 1-octanol and different specific isotopic labeled standards were used for LLE-SAFE analysis. Comparing obtained concentrations of different analytical methods would not be expressive. A closer explanation is given in **4.1.2**. Only 2,5-dimethylpyrazine can confirm the conclusion. In general, it was detectable by headspace as well as by LLE-SAFE analysis. While it was found in small amounts in every sample of the milk protein/glucose syrup design when prepared by extraction/distillation, it could not be detected by headspace analysis.

After all, none of the used preparation techniques showed definite advantages against the other regarding a complete picture of generated flavor compounds. It depends on the individual demands which technique is to prefer over the other. However, regarding time consumption and effectiveness, headspace sampling remains the technique of choice.

4.1.2 Quantification by Internal Standards Compared to Stable Isotope Dilution Analysis (SIDA)

Quantification by common internal standards was compared to the relatively new method of stable isotope dilution analysis. The aim was to investigate in how far results obtained by the use of common internal standards differ from the most accurate results obtained by isotopic labeled reference compounds. As mentioned in 2.4.3.2, isotopomeres of investigated flavor compounds were used. As well as the common standard they were added in fixed amounts to the sample at the beginning of the preparation process. Unlike usual standards, isotopomeres react the same way as their comparable analytes. By determining the concentrations of the investigated flavor compound and its dedicated isotopomere, an accurate quantification was possible. However, it has to be considered that isotopic labeled standards are not available on the market for every chemical compound.

Beside isotopic standards, 4-heptanone and 1-octanol were used as usual standards. Both were selected since they did not occur naturally in the sample material. Furthermore, they were easily detectable by GC/MS and did not interfere with other compounds. 4-heptanone and 1-octanol were used for quantification of all detected compounds. The degree of loss during sample preparation is different for every compound. Therefore, only relative changes of analyte concentrations could be described, referring to the internal standard.

As a compromise between isotopic labeling and the use of a one and only internal standard, unlabeled trimethylpyrazine was added. It did not appear in detectable concentrations in the original samples and was used for quantification of pyrazines only. An expressive evaluation was possible since all pyrazines react almost in the same way during sample preparation. Thus, losses could have been ideally compensated. Differences in real concentrations, found by stable isotope dilution analysis and quantification by trimethylpyrazine (TMP), and estimated concentrations, referring to 1-octanol, are shown in **Table 13** for samples containing milk protein and glucose syrup, using the example of methylpyrazine, 2,5-dimethylpyrazine, and butyric acid. The factors indicate the deviations of 1-octanol from the corresponding specific standard.

It was found that quantifications made by stable isotope dilution analysis, or rather TMP, and common internal standards showed significant variations between the calculated concentrations. Higher concentrations were detected when the evaluation was based on the internal standard 1-octanol. Excluding the outliers, methylpyrazine and 2,5-dimethylpyrazine showed 4 to 8 times higher concentrations compared to evaluation related to TMP. Less pronounced variations were found for butyric acid at factors ranging from 1.3 to 2.4. The stronger variation of factors ranging from 4 to 8 for pyrazines is explainable differently. On the one hand it is due to the reaction behavior during sample

preparation. Thereby, the analytical loss of pyrazines is different to those of 1-octanol which was found to be more similar to butyric acid, resulting in minor variations. On the other hand, pyrazines are present in smaller amounts. Quantification was made by comparing the peak area of the compound of interest with the one of the standard. Thus, the smaller the peak area, the bigger is the chance of stronger variations due to higher impacts of inexact calculations.

Table 13: Comparison of concentrations of selected compounds in extrudate samples produced according to a milk protein/glucose syrup design, determined by TMP or SIDA and 1-octanol

Sample			Methylpyrazine [ppm]			2,5-Dimethylpyrazine [ppm]			Butyric Acid [ppm]		
Temp. [°C]	Protein [%]	Sugar [%]	TMP	1-Octanol	**Factor**	TMP	1-Octanol	**Factor**	SIDA	1-Octanol	**Factor**
140	1.3	2	0.36	1.94	**5.4**	0.08	0.41	**5.1**	14.10	23.50	**1.7**
	1.3	6	0.00	0.00		0.00	0.00		12.80	1.11	**0.1**
	2.6	2	0.28	2.33	**8.3**	0.06	0.54	**9.0**	3.00	7.25	**2.4**
	2.6	6	0.30	1.90	**6.3**	0.06	0.35	**5.8**	1.90	4.37	**2.3**
160	0	4	0.12	0.70	**5.8**	0.02	0.13	**6.5**	2.70	3.83	**1.4**
	1.3	4	0.39	1.68	**4.3**	0.10	0.44	**4.4**	25.40	46.70	**1.8**
	2	0	0.57	3.29	**5.8**	0.12	0.69	**5.8**	11.70	49.25	**4.2**
	2	4	0.57	4.04	**7.1**	0.10	0.73	**7.3**	20.10	67.50	**2.3**
	2	8	0.32	2.61	**8.2**	0.06	0.48	**8.0**	9.90	46.25	**4.7**
	2.6	4	0.35	2.46	**7.0**	0.07	0.53	**7.6**	5.00	11.43	**2.3**
180	1.3	2	0.63	2.72	**4.3**	0.15	0.66	**4.4**	26.20	36.64	**1.4**
	1.3	6	0.14	0.60	**4.3**	0.04	0.15	**3.8**	2.60	3.46	**1.3**
	2.6	2	0.70	2.09	**3.0**	0.17	0.70	**4.1**	22.50	38.87	**1.7**
	2.6	6	0.46	2.34	**5.1**	0.09	0.40	**4.4**	27.4	47.02	**1.7**
Average Factor					**5.8**			**5.9**			**2.3**

SIDA - stable isotope dilution analysis
TMP – trimethylpyrazine
outliers marked in grey

As can be seen in **Figure 19**, quantification by trimethylpyrazine as well as by 1-octanol showed similar curve progressions for the formation of pyrazines depending on different protein concentrations. An additional analysis of variance was made to check on the stability of the results obtained. This was necessary to see whether variations which are due to the use of 1-octanol could be easily compensated by including a compensative factor or not. The results are depicted for methylpyrazine in **Figure 20** and for 2,5-dimethylpyrazine in **Figure 21**. Analysis was made using a linear model as it promised the most appropriate fit and was suggested by DesignExpert®. Comparisons of the coefficients of determination (R^2) showed higher stabilities when using TMP as quantification standard. Its stability was found to be 70% higher when quantifying

methylpyrazine and 14% higher when quantifying 2,5-dimethylpyrazine. By that, the use of a compensative factor can be ruled out.

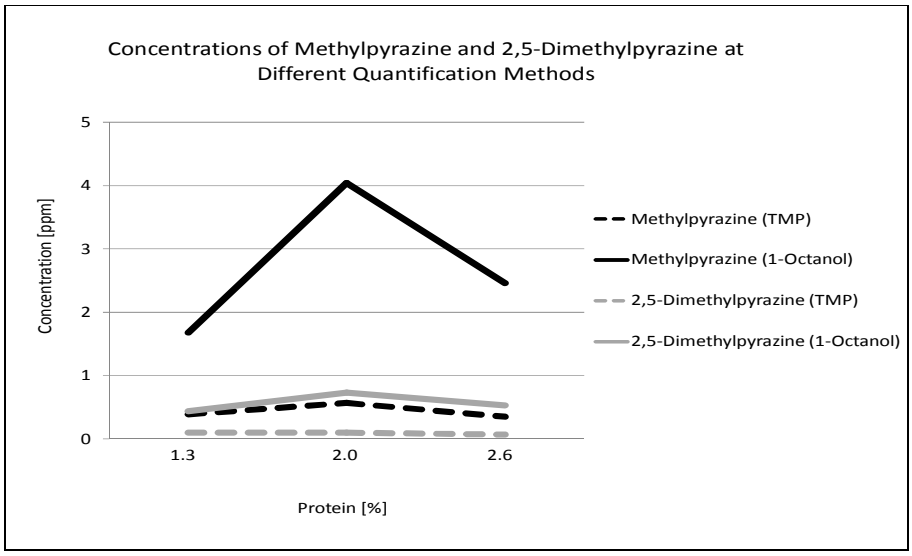


Figure 19: Influence of different quantification methods on the determined concentration of methylpyrazine and 2,5-dimethylpyrazine in milk protein/glucose syrup samples containing 4% sugar and differing protein content, processed at 160°C.

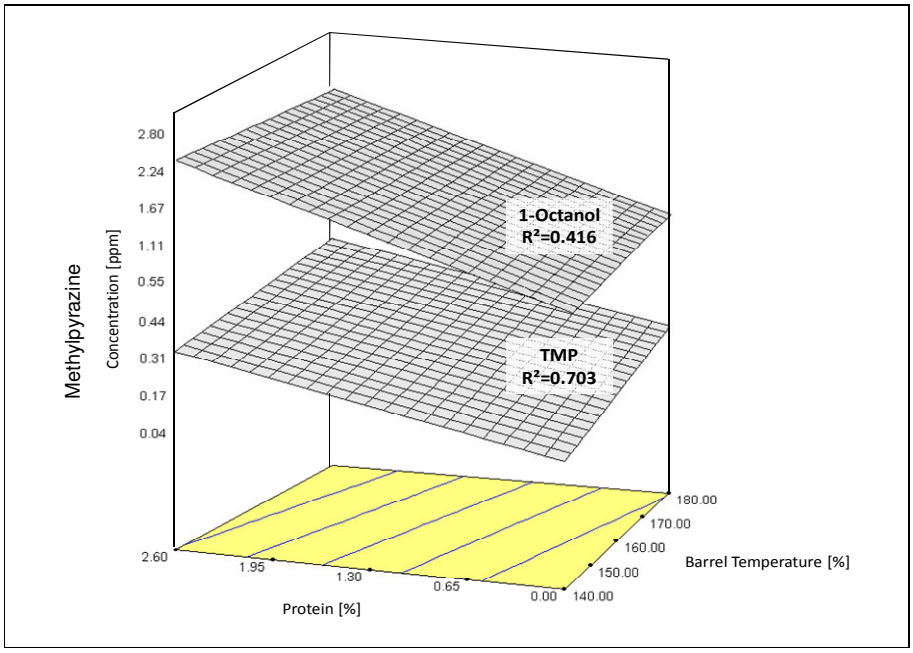


Figure 20: Comparison of methylpyrazine concentration in milk protein/glucose syrup samples containing 4% glucose syrup obtained by quantification using 1-octanol and trimethylpyrazine (TMP).

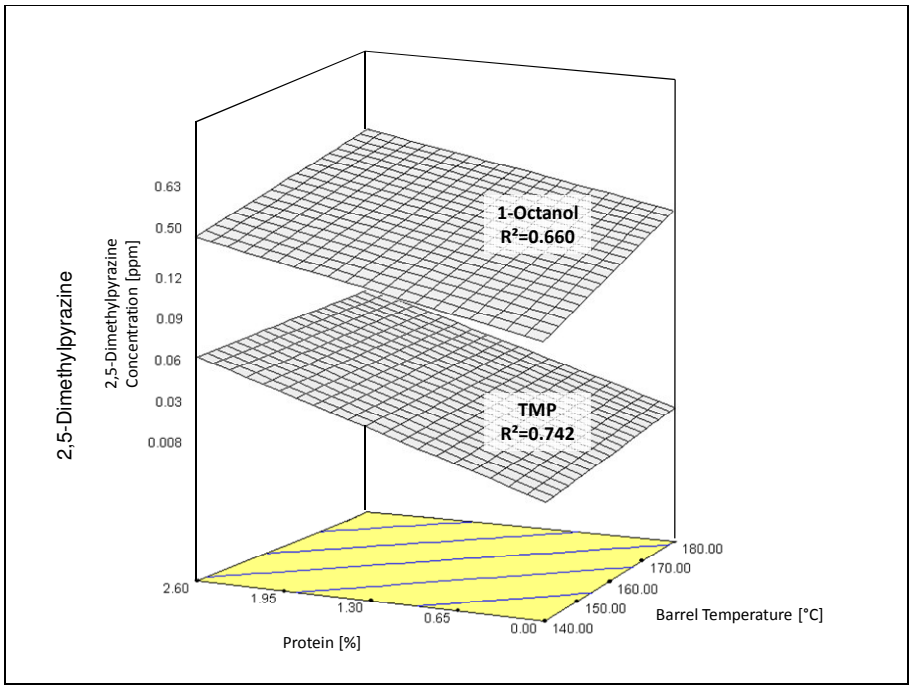


Figure 21: Comparison of 2,5-dimethylpyrazine concentration in milk protein/glucose syrup samples containing 4% glucose syrup obtained by quantification using 1-octanol and trimethylpyrazine (TMP).

Butyric acid is affected differently. Its loss during sample preparation and analysis is more similar to that of 1-octanol, resulting in lower variations with an average factor of 2.3. That means that evaluation showed about twice as much butyric acid when quantification was made by comparing the peak area of butyric acid with the one of 1-octanol than with the one of isotopic ^{13}C-butyric acid. **Figure 22** shows the development of butyric acid concentration at varied concentrations of milk protein for both quantification approaches. Again, similar curve progressions were found.

As for the examined pyrazines, analysis of variance was made to look for the stability of both attempts of quantification. This time a quadratic model was suggested and chosen, promising the most appropriate fit.

The obtained coefficients of determination, as can be seen in **Figure 23**, were found to be identical with R^2=0.740 using SIDA, or rather R^2=0.742 using 1-octanol. Thus, in case of butyric acid, stable isotope dilution analysis did not show advantages over the common standard 1-octanol regarding statistic stability of the obtained quantification results. Differences in the calculated amounts of butyric acid, using 1-octanol as standard, can be corrected by a recalculation using the compensation factor.

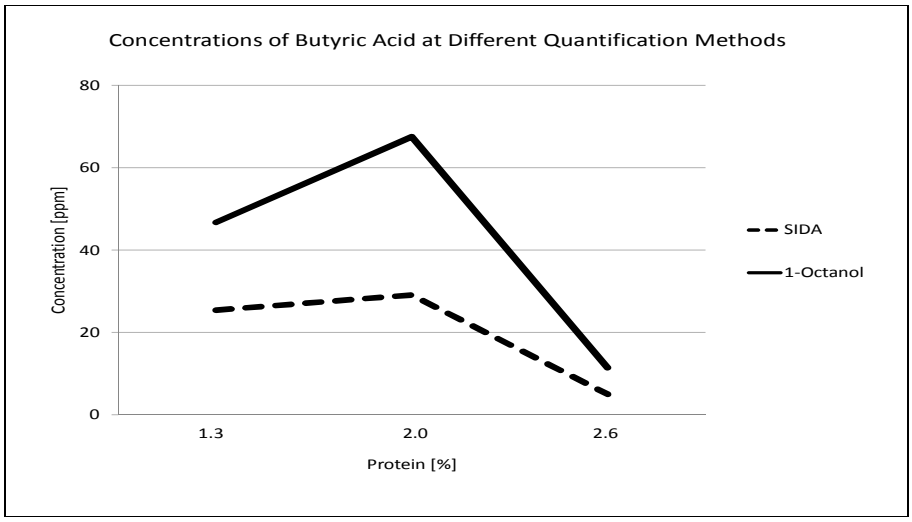


Figure 22: Influence of different quantification methods on the determined concentration of butyric acid in milk protein/glucose syrup samples containing 4% sugar and differing in protein content, processed at 160°C.

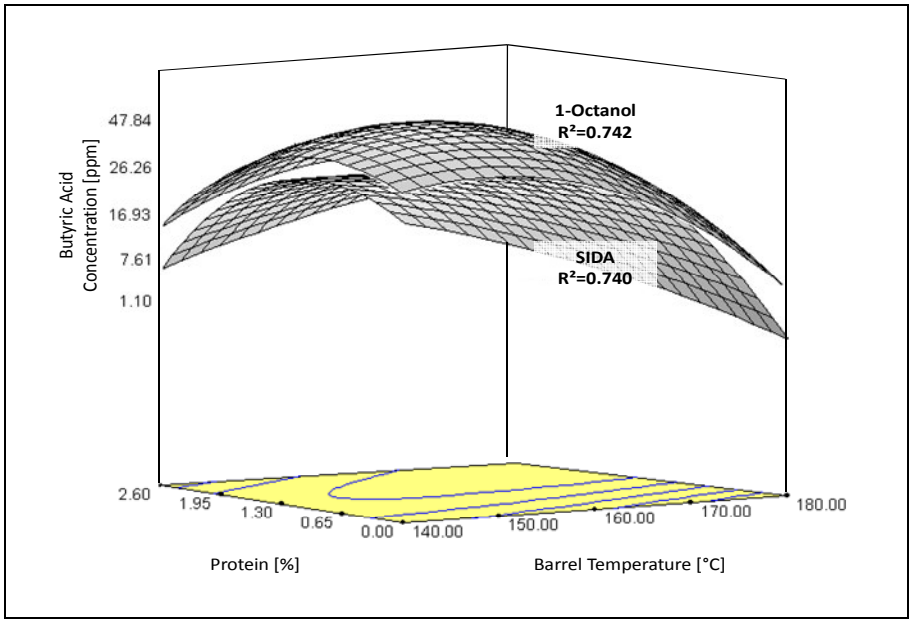


Figure 23: Comparison of butyric acid concentration in milk protein/glucose syrup samples containing 4% glucose syrup quantified by using the standards 1-octanol and C^{13} labeled butyric acid (stable isotope dilution analysis, SIDA).

Summarizing it can be concluded that the use of conventional internals standards can help determine general effects caused by different process conditions or ingredient compositions. However, an accurate quantification as it is often necessary in flavor analysis is difficult. It was found that the use of a compensating factor becomes possible if analysis of variance shows high and comparable coefficients of determination. How-

ever, such a factor has to be calculated for each compound individually every time a new sample preparation technique or a new GC/MS is used. Precious data can only be obtained immediately by using SIDA or selected chemical compounds. As mentioned previously, the latter must not naturally occur in the sample material and have to show the same reactive behaviors and losses during sample preparation and analysis as the examined compounds. Ideally, they should be closely related to the examined compound. Finding such compounds is complicated, making trimethylpyrazine as it was used within this study a rare exception. Its use was possible due to the fact that the analyzed samples within this model study contained starch, sugar, and protein only. The varieties and total amounts of flavor compounds were comparatively low. Traditionally extruded cereal products, containing whole flours, would show a multiple.

4.2 Influence of Process Parameters on Product Parameters

Eight aroma potent compounds were selected and analyzed regarding their formation depending on changeable process parameters.

Methylpyrazine and 2,5-dimethylpyrazine were analyzed since their appearance is of the utmost importance for roasted and toasted flavor impressions. Furthermore it is well known that the Maillard reaction is an important pathway for generating pyrazines and their formation is positively influenced by protein and high temperatures. The same applies to maltol, which provides a caramel-like sweet odor and, depending upon its concentration, a bread-like taste. Additionally, maltol acts as a flavor enhancer, primarily intensifying sweetness. Furanmethanol is a typical sugar degradation product described in literature as temperature dependent with a slightly caramel-like up to burning odor [59]. 2,4-decadienal are breakdown products of fatty acids and indicators of oxidation processes. The influence on the formation of butyric acid, pentanoic acid, and hexanoic acid, as representatives of carboxylic acids, was of interest since they might contribute to the overall flavor of the final product. Seen alone, carboxylic acids represent off-flavor compounds providing fatty-rancid odors.

For broad and expressive evaluations, an accurate knowledge of the concentration within the sample is necessary. Beside maltol, all analyses and evaluations are based on quantification results obtained by the use of SIDA or TMP as internal standards. Maltol was quantified with the help of the internal standard 1-octanol. Because of the previously mentioned disadvantages of unspecific standards, the obtained results can only provide tendencies in how changeable parameters influence then formation of maltol without giving precious concentrations. The results for the 3 designs using whey protein/glucose, milk protein/glucose and milk protein/glucose syrup are shown in **Table 14** to **Table 16**. The statistical analyses regarding the influential properties of changeable process parameters are given in **Table 17** to **Table 19**.

Table 14: Concentration of selected flavor compounds in extrudate samples produced according to a whey protein/glucose design, all results in ppm

Trial	Temperature [°C]	Protein [%]	Sugar [%]	Methyl-pyrazine	2,5 Dimethyl-pyrazine	Furan-methanol	Maltol	2,4-Decadienal			Butyric Acid	Pentanoic Acid	Hexanoic Acid
								E,E	E,Z	total			
Whey Protein/Glucose	140	1.3	2	0.66	0.21	2.16	0.93	0.70	3.76	4.45	25.94	5.65	4.92
	140	1.3	6	0.42	0.11	1.61	0.57	1.20	3.73	4.92	31.40	8.01	8.06
	140	2	4	0.56	0.24	2.24	1.17	0.68	2.84	3.53	27.05	6.24	5.73
	140	2.6	2	0.81	0.40	3.17	1.92	0.26	1.84	2.10	25.61	5.73	5.68
	140	2.6	6	0.58	0.17	1.87	0.47	0.58	3.13	3.71	25.23	5.58	4.27
	160	0	0	0.31	0.14	4.64	0.66	2.60	9.21	11.82	24.48	5.22	4.42
	160	0	4	0.41	0.05	1.31	0.00	4.31	7.13	11.44	29.68	3.77	3.20
	160	1.3	4	1.69	0.23	2.30	0.53	1.17	4.23	5.39	28.94	2.78	2.08
	160	2	0	0.77	0.58	8.67	2.59	0.66	3.62	4.28	21.83	4.02	3.91
	160	2	4	2.08	0.32	2.91	0.76	1.51	5.84	7.34	26.05	2.58	1.56
	160	2	8	0.68	0.09	1.53	0.75	1.64	5.41	7.06	24.52	2.58	2.05
	160	2.6	4	2.09	0.35	3.12	1.23	0.68	3.06	3.74	27.68	2.48	1.87
	180	1.3	2	1.14	0.40	3.47	2.54	1.01	4.35	5.36	31.14	7.40	7.50
	180	1.3	6	0.78	0.18	2.21	0.77	1.24	3.45	4.69	27.02	5.40	4.72
	180	2	4	1.14	0.39	3.61	1.95	0.73	2.35	3.08	28.60	6.23	5.92
	180	2.6	2	1.76	0.76	6.24	3.32	0.34	1.83	2.17	28.43	5.73	5.74
	180	2.6	6	0.83	0.19	2.56	1.15	0.39	1.91	2.29	27.80	6.40	5.70

Table 15: Concentration of selected flavor compounds in extrudate samples produced according to a milk protein/glucose design, all results in ppm

Trial	Temperature [°C]	Protein [%]	Sugar [%]	Methyl-pyrazine	2,5 Dimethyl-pyrazine	Furan-methanol	Maltol	2,4-Decadienal			Butyric Acid	Pentanoic Acid	Hexanoic Acid
								E,E	E,Z	total			
Milk Protein/Glucose	140	1.3	2	0.59	0.15	7.83	5.27	1.65	4.31	5.97	31.61	8.98	10.03
	140	1.3	6	0.28	0.07	3.82	2.89	2.40	4.86	7.25	32.41	9.67	9.70
	140	2	4	0.47	0.11	18.27	5.89	1.47	3.54	5.01	30.01	7.61	8.27
	140	2.6	2	0.50	0.15	20.15	9.80	0.85	3.01	3.87	28.85	6.60	7.23
	140	2.6	6	0.58	0.09	8.12	5.53	0.99	2.99	3.98	27.17	5.20	6.10
	160	0	4	0.68	0.08	1.75	2.48	4.09	6.91	11.00	23.45	2.90	1,83
	160	1.3	4	1.21	0.16	6.64	2.42	2.23	6.21	8.45	28.99	3.66	2.76
	160	2	0	0.49	0.13	35.70	11.06	1.05	3.31	4.36	28.40	5.18	5.67
	160	2	4	1.35	0.17	12.13	2.90	1.59	4.58	6.18	29.24	3.54	2.78
	160	2	8	0.64	0.09	4.99	1.12	2.38	6.08	8.46	24.24	3.98	3.93
	160	2.6	4	1.19	0.19	15.40	3.69	1.46	4.45	5.91	28.42	3.55	3.59
	180	1.3	2	0.65	0.22	12.56	5.45	1.29	4.23	5.52	28.38	6.74	5.61
	180	1.3	6	0.48	0.11	4.99	2.21	1.37	3.78	5.15	29.98	9.70	8.82
	180	2	4	0.78	0.21	22.95	7.05	1.01	3.45	4.47	28.80	7.49	6.92
	180	2.6	2	1.45	0.36	56.18	15.87	0.73	2.74	3.47	29.83	6.98	7.10
	180	2.6	6	0.64	0.18	17.58	4.87	0.84	2.90	3.74	26.99	6.55	6.17

Table 16: Concentration of selected flavor compounds in extrudate samples produced according to a milk protein/glucose syrup design, all results in ppm

Trial	Temperature [°C]	Protein [%]	Sugar [%]	Methyl-pyrazine	2,5 Dimethyl-pyrazine	Furan-methanol	Maltol	2,4-Decadienal			Butyric Acid	Pentanoic Acid	Hexanoic Acid
								E,E	E,Z	total			
Milk Protein/Corn Syrup	140	1.3	2	0.36	0.08	8.13	3.14	1.09	3.61	4.70	14.15	2.83	7.78
	140	1.3	6	0.00	0.00	7.09	1.42	0.95	3.84	4.79	12.78	26.32	326.32
	140	2	4	0.21	0.05	10.30	3.33	1.02	3.46	4.48	7.97	7.97	90.76
	140	2.6	2	0.28	0.06	16.42	5.88	0.96	3.64	4.59	3.05	1.84	21.05
	140	2.6	6	0.30	0.06	9.41	2.83	1.01	3.25	4.26	1.90	0.89	7.91
	160	0	4	0.12	0.02	1.35	0.46	3.03	6.45	9.48	2.71	1.05	4.82
	160	1.3	4	0.39	0.10	12.48	9.08	1.88	6.97	8.85	25.43	4.72	5.88
	160	2	0	0.57	0.12	27.78	11.13	0.97	3.33	4.31	11.65	1.82	2.20
	160	2	4	0.57	0.10	16.04	14.28	1.75	6.34	8.09	29.10	5.31	6.04
	160	2	8	0.32	0.06	7.29	2.75	1.49	4.52	6.01	9.94	1.88	2.91
	160	2.6	4	0.35	0.07	17.79	4.72	0.79	2.85	3.64	4.97	1.17	6.64
	180	1.3	2	0.63	0.15	19.26	9.52	1.11	4.14	5.25	26.22	5.56	5.65
	180	1.3	6	0.14	0.04	4.65	2.61	1.60	5.01	6.61	2.55	1.42	15.06
	180	2	4	0.48	0.11	18.90	6.53	1.00	3.84	4.84	19.65	4.21	8.12
	180	2.6	2	0.70	0.17	31.45	9.26	0.66	3.06	3.72	22.49	4.10	5.23
	180	2.6	6	0.46	0.09	19.91	4.79	0.75	3.15	3.90	27.36	5.77	6.55

Table 17: Regression coefficients and coefficients of determination for the influence of process parameters on selected flavor compounds using whey protein and glucose

Source	Factor	Methyl pyrazine	2,5-Dimethyl pyrazine	Furan methanol	Maltol	2,4-Decadienal	Butyric Acid	Pentanoic Acid	Hexanoic Acid
Model	-	**+1.83**[2)]	**+0.32**[3)]	**+3.16**[1)]	**+0.94**[2)]	**+6.23**[1)]	+26.28	**+2.70**[2)]	**+1.87**[1)]
Linear	A	**+0.26**[1)]	**+0.079**[3)]	**+0.70**[2)]	**+0.47**[3)]	-0.11	+0.78	-0.0043	+0.091
	B	+0.14	**+0.075**[3)]	**+0.52**[1)]	**+0.28**[2)]	**-1.08**[1)]	-0.97	**-**0.33	-0.40
	C	-0.18	**-0.14**[3)]	**-1.04**[2)]	**-0.66**[3)]	+0.29	+0.17	+0.017	-0.20
Quadratic	A^2	**-0.86**[2)]	+0.0012	-0.36	**+0.53**[1)]	**-2.38**[2)]	+1.43	**+3.49**[3)]	**+3.80**[2)]
	B^2	+0.18	-0.023	-0.58	-0.15	-1.11	+1.92	-0.12	-0.049
	C^2	-0.31	+0.011	+0.72	+0.17	+0.83	-1.78	+0.19	+0.24
Interactive	AB	+0.044	+0.014	+0.23	+0.032	-0.25	+0.57	+0.21	+0.28
	AC	-0.10	**-0.058**[2)]	-0.39	**-0.27**[1)]	-0.33	**-1.23**[1)]	-0.44	-0.57
	BC	-0.071	**-0.059**[2)]	-0.40	-0.19	+0.24	-0.29	**+**0.020	-0.23
Coefficient of Determination		0.9350	0.9816	0.9209	0.9660	0.8919	0.8162	0.9284	0.8941

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)

A=barrel temperature, B=protein, C=sugar

Table 18: Regression coefficients and coefficients of determination for the influence of process parameters on selected flavor compounds using milk protein and glucose

Source	Factor	Methyl pyrazine	2,5-Dimethyl pyrazine	Furan methanol	Maltol	2,4-Decadienal	Butyric Acid	Pentanoic Acid	Hexanoic Acid
Model	-	+1.19	**+0.16**[2)]	**+14.12**[2)]	**+3.38**[2)]	**+6.45**[2)]	**+28.69**[1)]	**+3.71**[2)]	**+3.17**[2)]
Linear	A	+0.16	**+0.052**[2)]	**+5.61**[1)]	+0.61	-0.37	-0.61	-0.059	-0.67
	B	+0.12	**+0.027**[3)]	**+8.16**[2)]	**+2.15**[2)]	**-1.14**[3)]	**-1.01**[2)]	**-0.99**[2)]	-0.67
	C	-0.11	**-0.046**[2)]	**-7.76**[2)]	**-2.59**[3)]	+0.34	-0.42	+0.12	-0.005
Quadratic	A^2	**-0.49**[1)]	+0.00251	+5.50	**-2.85**[1)]	**-1.85**[2)]	+0.99	**+3.77**[3)]	**+4.23**[3)]
	B^2	-0.085	+0.020	-4.09	-0.56	+0.59	+0.29	-0.18	-0.19
	C^2	-0.16	-0.019	+1.13	+0.88	-0.29	-0.63	+0.28	+0.43
Interactive	AB	+0.093	+0.024	**+4.95**[1)]	+0.74	+0.24	**+0.81**[1)]	+0.49	+0.65
	AC	-0.096	-0.020	-3.76	-0.95	-0.19	-0.046	+0.41	+0.47
	BC	-0.032	-0.006	**-4.88**[1)]	**-1.21**[1)]	-0.067	**-0.87**[1)]	**-0.69**[1)]	-0.62
Coefficient of Determination		0.8484	0.9308	0.9387	0.9416	0.9325	0.8879	0.9609	0.9430

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)
A=barrel temperature, B=protein, C=sugar

Table 19: Regression coefficients and coefficients of determination for the influence of process parameters on selected flavor compounds using milk protein and glucose syrup

Source	Factor	Methyl pyrazine	2,5-Dimethyl pyrazine	Furan methanol	Maltol	2,4-Decadienal	Butyric Acid	Pentanoic Acid	Hexanoic Acid
Model	-	**+0.49**[1)]	**+0.098**[1)]	**+16.01**[3)]	+11.23	+7.16	+22.74	+4.18	+5.62
Linear	A	**+0.13**[2)]	**+0.031**[1)]	**+4.28**[3)]	+1.61	+0.15	+5.48	-1.88	-41.32
	B	+0.054	+0.0079	**+4.37**[3)]	+0.17	**-1.01**[1)]	-2.14	-2.71	-31.3
	C	**-0.12**[2)]	**-0.031**[2)]	**-4.45**[3)]	-2.03	+0.21	-2.22	+2.01	+31.65
Quadratic	A^2	-0.11	-0.013	-1.40	**-4.78**[1)]	**-2.03**[1)]	-5.75	+2.47	+44.04
	B^2	-0.086	-0.006	-0.86	-2.81	-0.45	-4.36	-0.67	+0.85
	C^2	+0.05	+0.0007	+0.79	+0.90	-0.068	+0.39	-0.036	-1.11
Interactive	AB	+0.021	+0.003	**+2.11**[1)]	-0.28	-0.45	+5.38	+3.66	+37.03
	AC	-0.048	-0.014	**-2.26**[1)]	-0.83	+0.22	-2.03	-3.13	-36.83
	BC	**+0.080**[1)]	+0.013	-0.36	+0.14	+0.20	+3.59	-2.33	-42.47
Coefficient of Determination		0.9120	0.9199	0.9727	0.8137	0.7744	0.7196	0.7513	0.8164

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)
A=barrel temperature, B=protein, C=sugar

The statistical evaluation showed distinct differences in how process parameters affect the formation of different flavor compounds. Those differences will be displayed, analyzed, and evaluated in detail on selected flavor compounds within the following chapters.

4.2.1 Influences on Pyrazine Formation

The group of pyrazines, represented by methylpyrazine and 2,5-dimethylpyrazine, was found to be significantly influenced by increasing temperatures. As can be seen in **Figure 26**, the amount of methylpyrazine rose intensely when temperature was increased from 140°C to 160°C. While already starting with the highest concentration at 140°C, the rate of increase for whey protein/glucose samples was around 400%. Milk protein samples still showed a 1.5 times higher concentration of methylpyrazine at 160°C compared to those generated at 140°C. A further elevation of temperature to 180°C led to a decreasing methylpyrazine formation. Whereas higher concentrations at 160°C were expected and were in line with reviewed literature [31, 44], the detected decrease at 180°C has not been reported yet. A possible reason is a higher degree of degradation processes, leading to the formation of other chemical compounds.

This effect was exclusively to be found for methylpyrazine. 2,4-dimethypyrazine showed the expected increase at 160°C as well as at 180°C. (**Figure 27**) Again, this general trend was independent from the protein or sugar used, with approximately 2 times higher amounts at 180°C compared to those at 140°C. However, the kind of protein and sugar played an important role for the total amounts of detected 2,5-dimethylpyrazine. As can be seen in **Figure 24**, comparing analyses of both designs using glucose clearly showed higher amounts when whey protein was used instead of milk protein. At all temperatures, their concentrations were about twice as high. Beside possible differences in the quantity of available nitrogen from the protein, necessary for nitrogen containing heterocyclic pyrazines, additional sugar from the protein source milk powder has to be taken into account. The free carbonyl groups of reducing sugars easily react with free nitrogen, generating a broad spectrum of chemical compounds beside pyrazines. Hence, higher degrees of sugar negatively influence the pyrazine formation.

Beside temperature and the kind of protein used, the formation of pyrazines was found to be influenced by the type of sugar as can be seen for 2,5-dimethylpyrazine in **Figure 25**. 2,5-dimethylpyrazine as well as methylpyrazine showed significantly higher degrees of generation when glucose was used instead of glucose syrup.

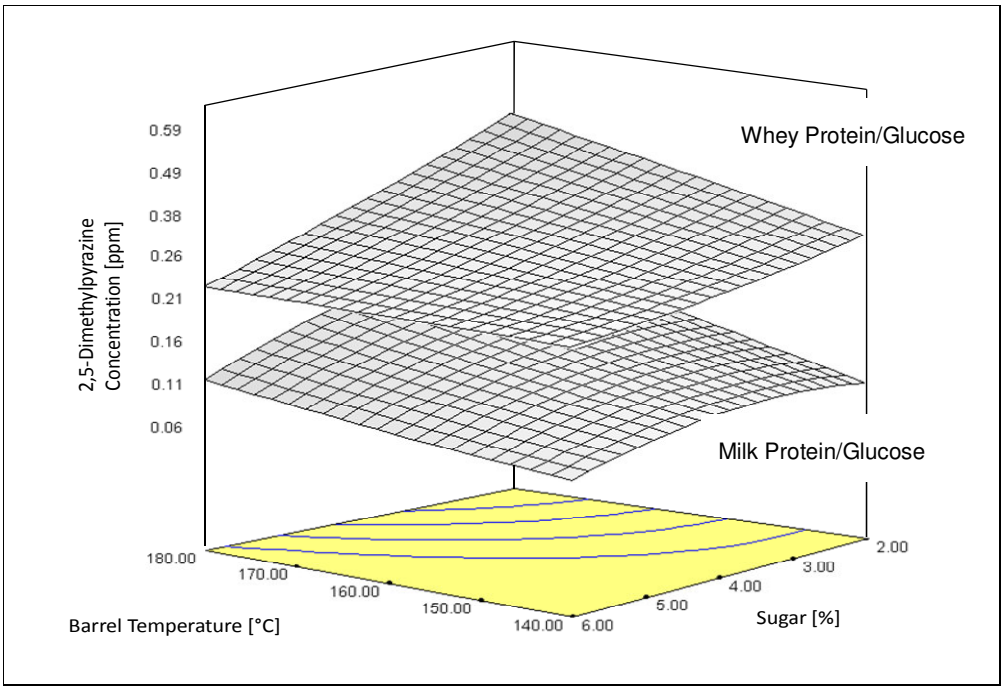


Figure 24: Influence of different proteins of constant concentration of 2% in the recipe on the formation of 2,5-dimethylpyrazine in dependence on sugar content and barrel temperature.

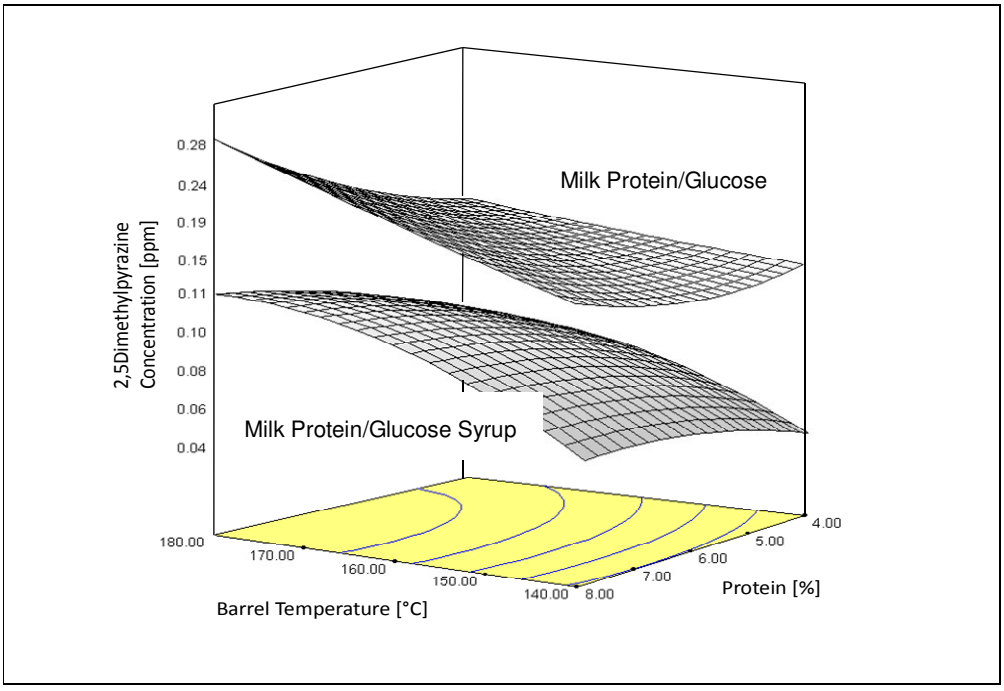


Figure 25: Influence of different sugars of constant concentration of 4% in the recipe on the formation of 2,5-dimethylpyrazine in dependence on protein content and barrel temperature.

Irrespective of the temperature, the amounts of pyrazine generated in glucose systems were about twice as high as when using glucose syrup due to its higher reducing power (as can be seen in **Figure 26** and **Figure 27**).

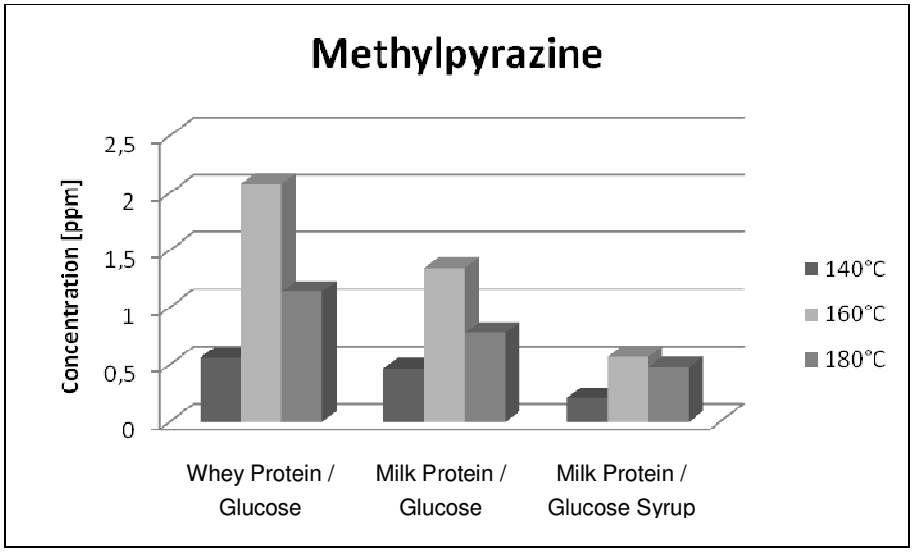


Figure 26: Influence of barrel temperature on methylpyrazine formation at constant protein (2%) and sugar (4%) contents.

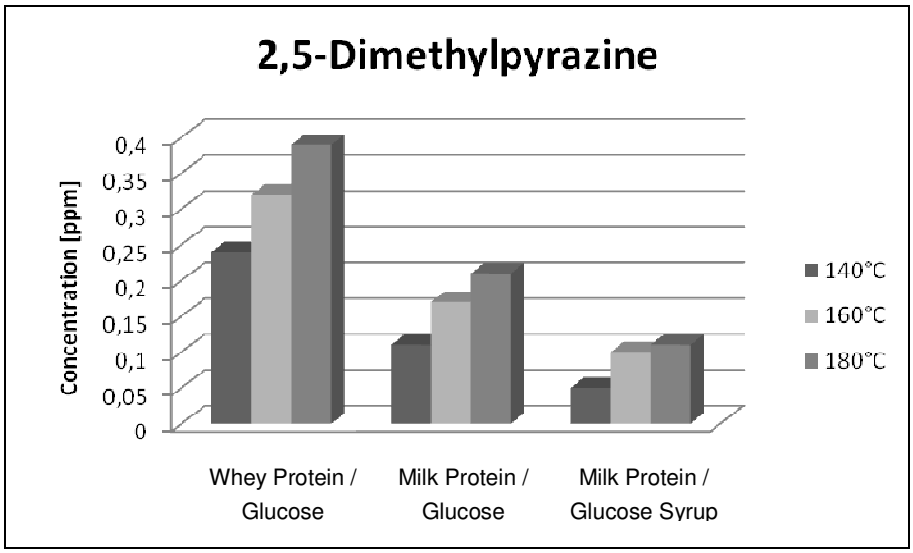


Figure 27: Influence of barrel temperature on 2,5-dimethylpyrazine formation at constant Protein (2%) and sugar (4%) contents.

Glucose as well as glucose syrup can act as catalysts for pyrazine formation within the Maillard reaction since both are, or rather contain, reducing sugars. Glucose syrup is a complex sugar, containing molecules of different length resulting in a limited reducing power of about 30% compared to glucose. In contrast, glucose only consists of single

glucose molecules, offering more reactive carbonyl groups. Hence, glucose favors the Maillard reaction at greater rates than glucose syrup and thereby the pyrazine generation.

The direct influence of different sugar contents on the formation of methylpyrazine and 2,5-dimethylpyrazine at 2% protein constantly for all designs is shown in **Figure 28** to **Figure 30**. All samples were extruded at 160°C.

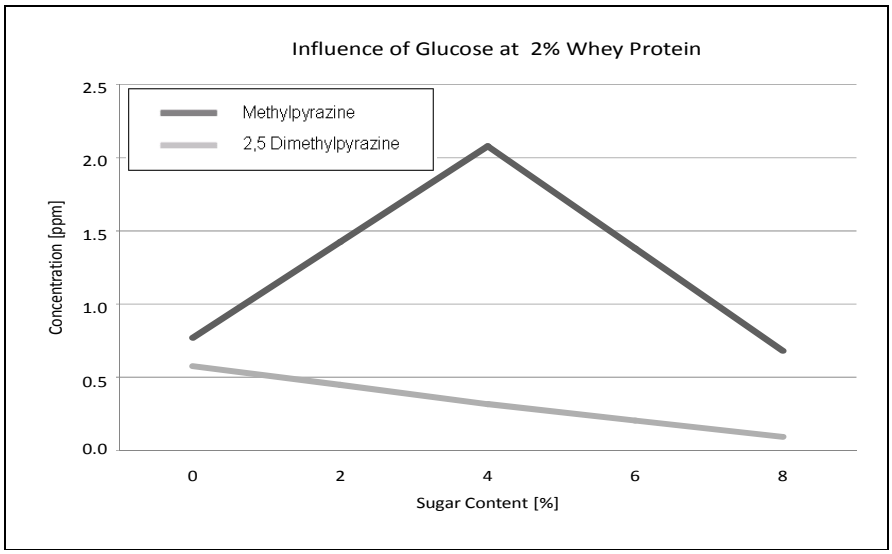


Figure 28: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of glucose at a constant whey protein content (2%), processed at 160°C.

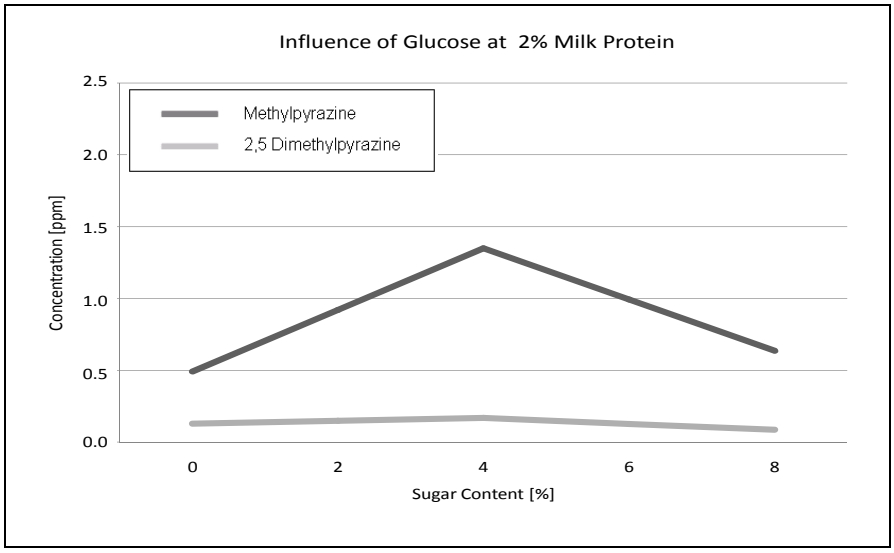


Figure 29: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of glucose at a constant milk protein content (2%), processed at 160°C.

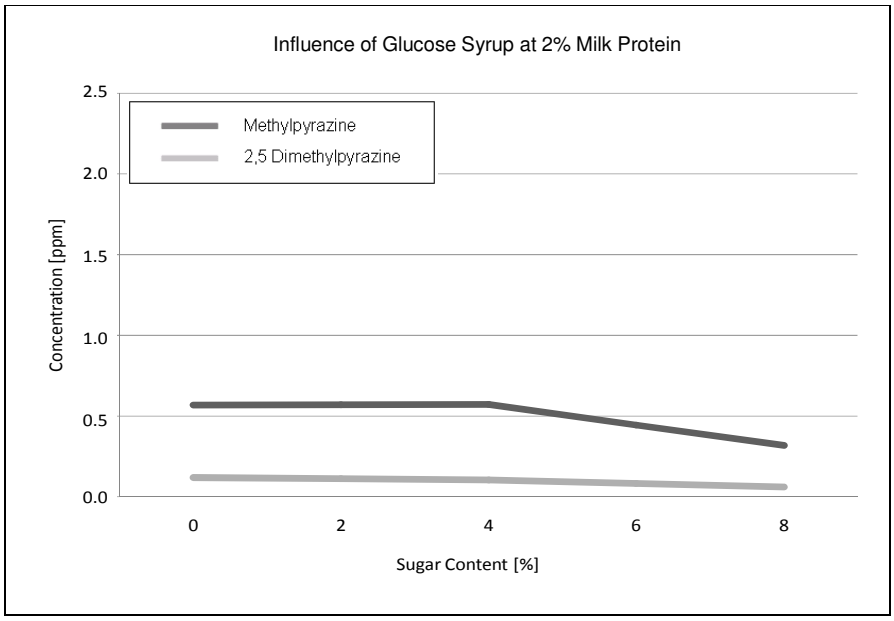


Figure 30: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of glucose syrup at a constant milk protein content (2%), processed at 160°C.

What becomes visible is the complex influence of sugar within the process of pyrazine formation. While 2,5-dimethylpyrazine formation is significantly negatively influenced by increasing amounts of sugar, methylpyrazine increased with glucose and remained constant with glucose syrup until reaching a total sugar concentration of 4%. Further increasing sugar contents resulted in an intensely decreasing methylpyrazine formation.

As mentioned previously, the most likely explanation can be found in an intensified consumption of nitrogen by the excess of free carbonyl groups of the sugar to form different nitrogen containing chemical compounds. Additionally, higher degrees of sugar tend to lower the extrusion temperature as explained in 4.3. However, this cannot be an explanation on its own since the decrease would be expected to be consistently.

The last changeable parameter was protein. Especially 2,5-dimethylpyrazine was significantly positively influenced by increasing amounts of whey as well as milk protein, whereas the influence on methylpyrazine formation did not show any statistical certainty due to inconsistent increases. (**Table 17** to **Table 19**)

The effect of increasing protein contents at 4% sugar constantly is shown in **Figure 31** to **Figure 33**.

Figure 31: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of whey protein at a constant glucose content (4%), processed at 160°C.

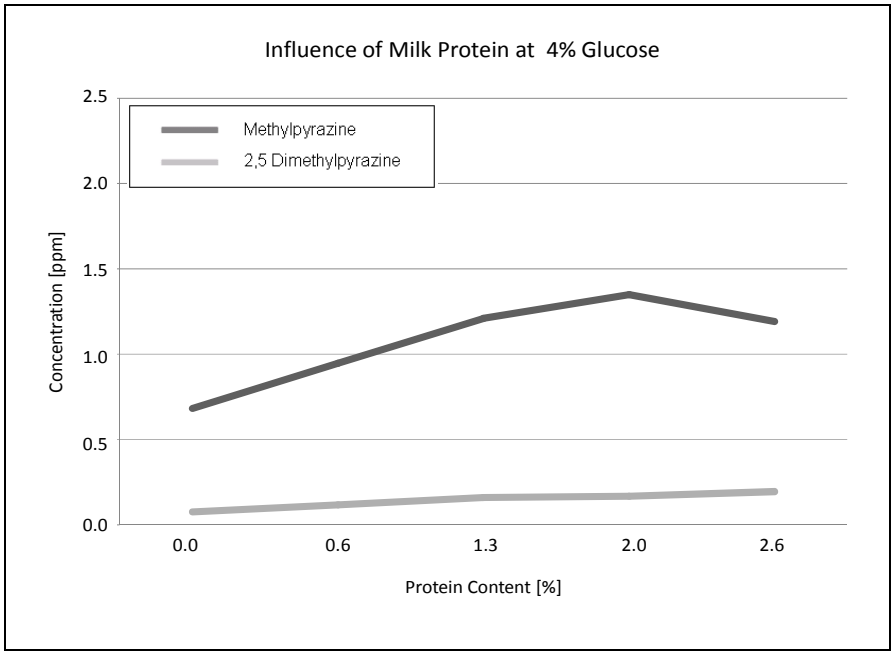


Figure 32: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of milk protein at a constant glucose content (4%), processed at 160°C.

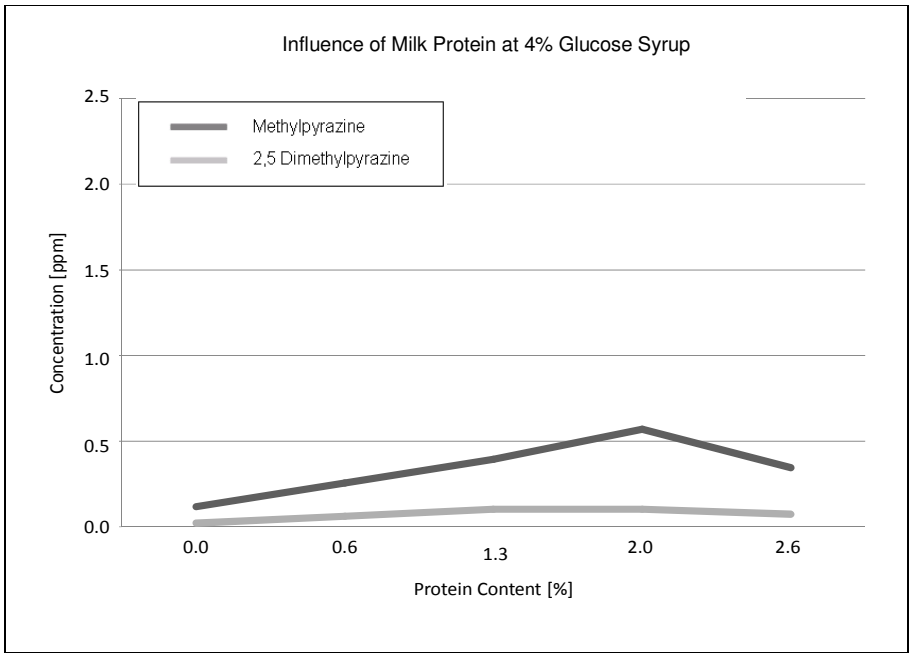


Figure 33: Concentration of methylpyrazine and 2,5-dimethylpyrazine depending on the amount of milk protein at a constant glucose syrup content (4%), processed at 160°C.

In contrast to statistical evaluation, increasing amounts of protein from 0 to 2.6% showed distinct influences on the formation of methylpyrazine as well as of 2,5-dimethylpyrazine. Whey protein showed the strongest impact on the total amounts as well as on the rate of increases compared to milk protein. When using whey protein, methylpyrazine quintupled from 0.41 ppm at 0% protein to 2.09 ppm at 2.6% protein while 2,5-dimethylpyrazine increased from 0.05 to 0.35 ppm, a sevenfold increase. Samples containing milk protein and glucose started at similar levels of pyrazines. However, the increase was only about 60% compared to that of whey protein samples. The methylpyrazine content reached its maximum with 1.35 ppm at 2%, followed by a decrease at 2.6% protein. The same applied to samples containing milk protein and glucose syrup. By quintupling the total amount of methylpyrazine, the maximum concentration was reached at 2% protein, followed by a decrease at 2.6%. The total increase in 2,5-dimthylpyrazine was similar to that of methylpyrazine but did not show that significant drop at 2.6% protein as methylpyrazine did in designs using milk protein.

Again, the abrupt decrease in methylpyrazine at 2.6% protein can be explained by the additional amounts of sugar from the protein source milk powder. At certain amounts, the excess of sugar leads to the formation of different nitrogen containing chemical compounds. Thus, less nitrogen remains to form pyrazines.

4.2.2 Influences on Furanmethanol Formation

Furanmethanol is a typical sugar degradation product, positively influenced by increasing temperatures. It plays a minor role as a flavor compound, but it was found that it could be produced by the Cannizzaro reaction from furfural, an even more important aroma compound itself, with a caramel and bread-like flavor [71]. Thus, higher amounts of furanmethanol may indicate higher furfural concentrations.

Furanmethanol was significantly influenced by all process parameters as can be seen in **Table 17** to **Table 19**. Temperature and protein positively influenced the formation of furanmethanol while elevated amounts of sugar led to significant decreases.

The amounts of generated furanmethanol depended strongly on the design or rather the sugar and protein used. Starting out at concentrations of about 1.5 ppm, detected in samples without any additional sugar or protein, whey protein/glucose samples reached a maximum concentration of 8.7 ppm, a nearly sevenfold increase. Replacing whey protein by milk protein led to an even more distinct increase of up to 56.2 ppm. This more than 6 times higher maximum value compared to whey protein showed the intense influence of different proteins. It has to be taken into account that sugar was found to significantly decrease the furanmethanol concentration. The fact that using milk powder as protein source increases the total sugar content, additionally underlines the impact of milk protein compared to whey protein. Replacing glucose by glucose syrup, using milk protein further on, resulted in still significant but less intense increases in furanmethanol formation. The maximum concentration was reached with 31.5 ppm, representing two fifth of the increase using glucose. A comparison of all 3 designs regarding the influence of sugar and protein at a constant barrel temperature of 160°C is shown in **Figure 34** and **Figure 35**.

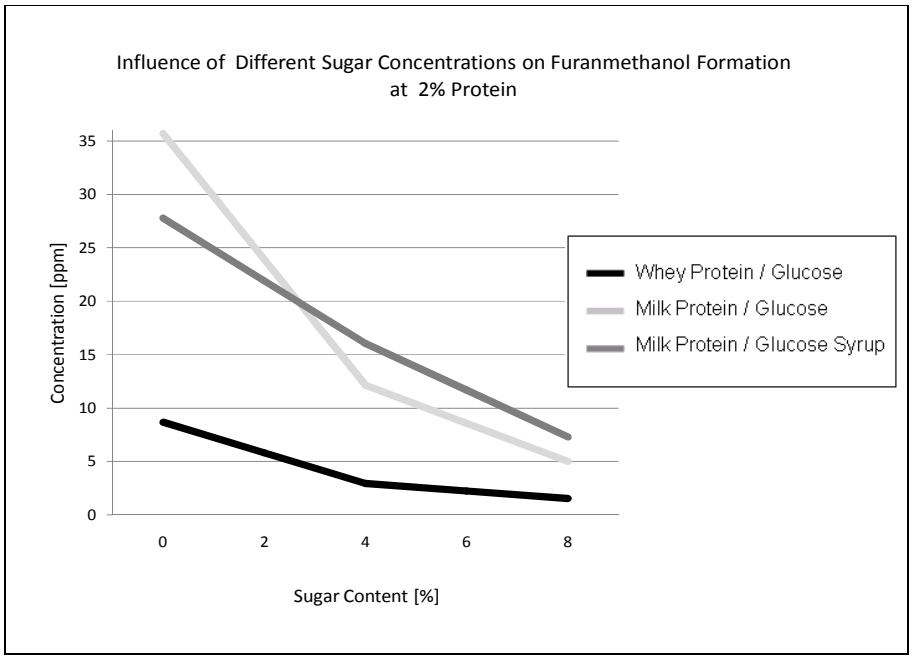


Figure 34: Concentration of furanmethanol depending on the amount of sugar at constant protein contents (2%), processed at 160°C.

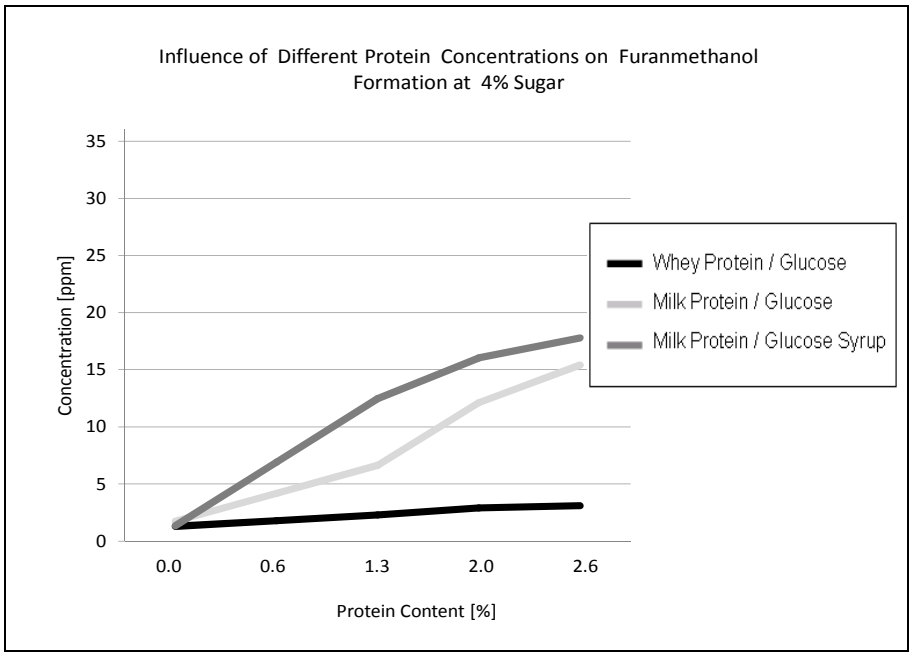


Figure 35: Concentration of furanmethanol depending on the amount of protein at constant sugar contents (4%), processed at 160°C.

Beside the influence of sugar and protein, temperature played an important and significant role in furanmethanol formation, as depicted in **Figure 36**.

No significant differences in the level of increase were detected from 140°C to 180°C comparing samples containing whey protein/glucose and milk protein/glucose syrup. The increase was about 70%. On the contrary, the use of milk protein in combination with glucose led to an increase of merely 25%. Additionally, an unexpected drop in furanmethanol concentration was detected at 160°C. This effect could not be explained. It must be assumed that this might be due to incorrect analytical results. The positive effect of elevated temperatures on furanmethanol remains definite and is thus in line with the common state of knowledge. As mentioned previously, furanmethanol may correlate with furfural concentrations. Both are found to be positively influenced by increasing temperature and amounts of protein [71]. However, more studies have to be carried out to confirm this assumption.

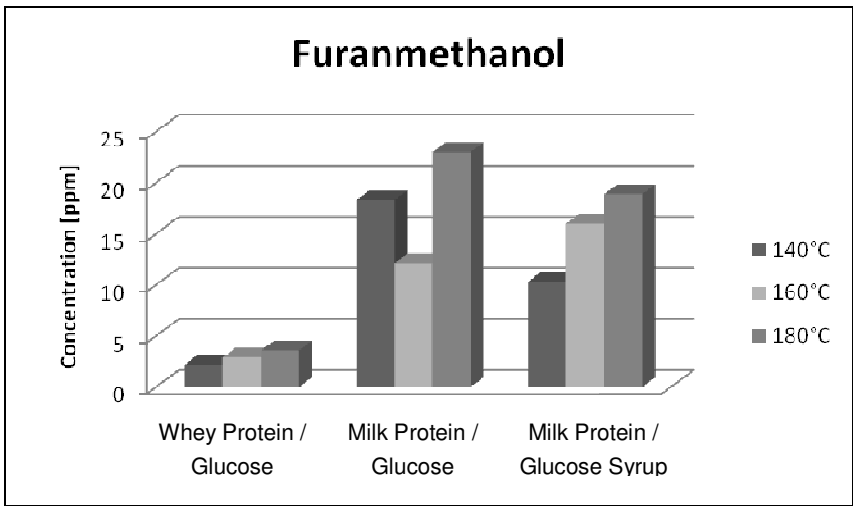


Figure 36: Influence of barrel temperature on furanmethanol formation at constant protein (2%) and sugar (4%) contents.

4.2.3 Influences on 2,4-Decadienal Formation

2,4-decadienal represents a fatty acid aldehyde, characterized by fatty and oily flavor impressions. It was found in extruded oat flour, responsible for typical off-flavor impressions [115]. The appearance of 2,4-decadienal indicates autoxidation. Thus, a lower concentration is favorable.

Analysis examined that 2,4-decadienal formation was negatively affected by increasing protein contents whereas modified amounts of sugar did not show a statistically proven influence. However, as can be seen in **Figure 37**, all designs showed an increase in 2,4-decadienal formation until 4% sugar. This increase continued for samples containing milk protein and glucose until reaching the final sugar content of 8%. The total concentration thus nearly doubled from 4.4 to 8.5 ppm. Samples containing combinations of whey protein and glucose as well as milk protein and glucose syrup, starting out with

the highest level of increase, showed a reduced 2,4-decadienal concentration at 6 and 8% sugar. This effect was found to be more pronounced in whey protein/glucose samples.

The results are ambiguous. An increase as it appeared until 4% sugar and as it continued in samples using milk protein and glucose was expected. Finding a conclusive reason for the decreasing concentration is difficult. From the chemical point of view, one may expect a more or less even increase of 2,4-decadienal at increasing sugar concentrations. This is due to radicals, formed from sugar by the so called Keto-Enol-Tautomerie and Fenton reaction. Those reactions are favored by ferric ions and oxygen, available during extrusion processing. Subsequently, free radicals start autoxidation processes, forming fatty acid peroxides and carbonyls such as 2,4-decadienal. Every attempted explanation would raise the question of why milk protein/glucose samples did not show the same behavior. After all, this question has to remain unanswered. However, it was found that sugar plays a part in the formation of 2,4-decadienal. Lowest concentrations are attainable by using a minimum of additional sugar.

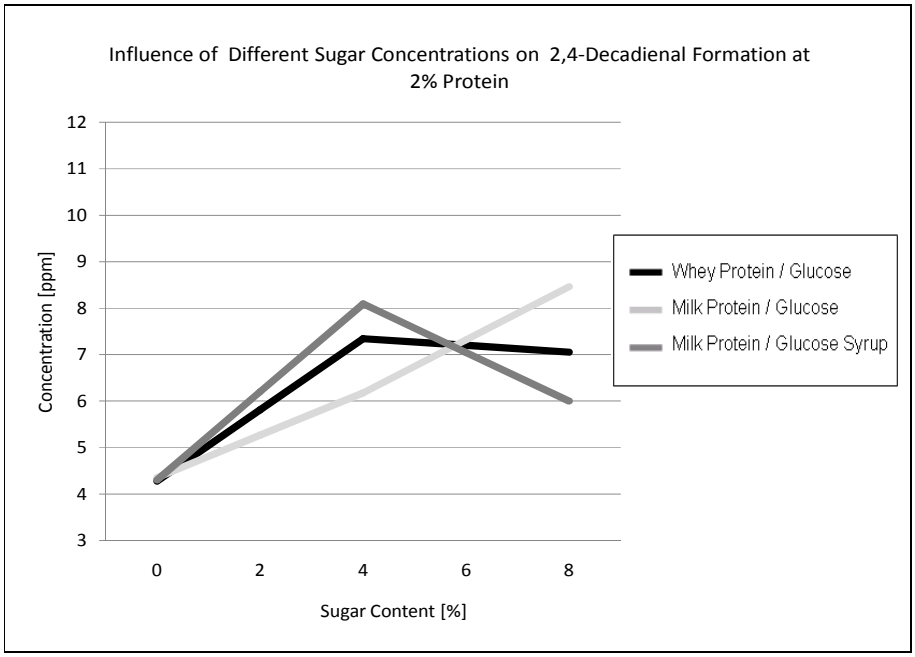


Figure 37: Concentration of 2,4-decadienal depending on the amount of sugar at constant protein contents (2%), processed at 160°C.

As mentioned previously, protein showed a significant negative influence on the formation of 2,4-decadienal. The concentrations as a function of protein contents between 0% and 2.6% at a barrel temperature of 160°C and a constant sugar concentration (4%) are depicted in **Figure 38** for all analyzed designs.

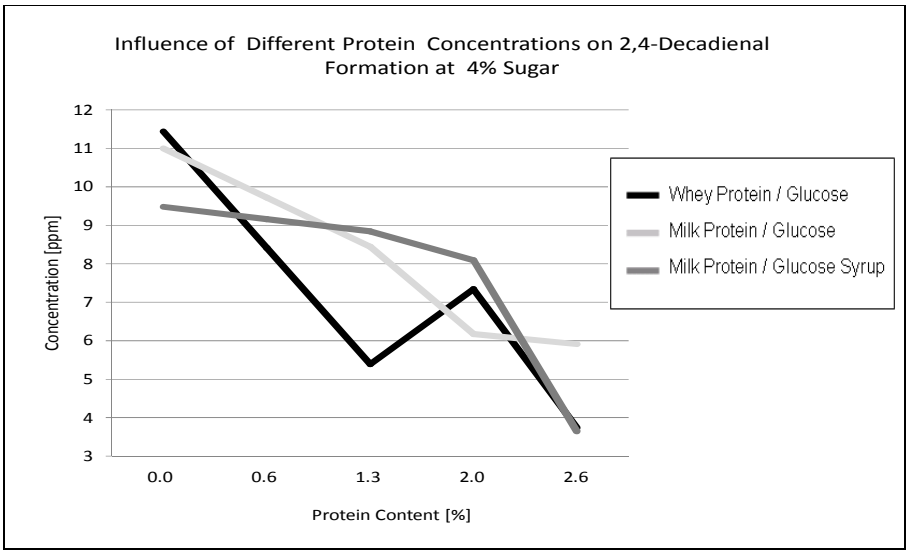


Figure 38: Concentration of 2,4-decadienal depending on the amount of protein at constant sugar contents (4%), processed at 160°C.

The changes in concentration were not as even as it was found for pyrazines or furanmethanol. However, the decrease was still distinct and statistically proven. Lower 2,4-decadienal concentrations at higher protein contents are due to a reduced availability of sugar for oxidation processes. More sugar is consumed by protein to form different chemical compounds such as pyrazines and furanmethanol. Thus, protein not only negatively affects the formation of 2,4-decadienal but also positively affects the development of different important aroma active compounds.

In contrast to sugar and protein, temperature showed a quadratic influence on the formation of 2,4-decadienal. This effect, visualized in **Figure 39** and **Figure 40**, was found to be independent from the sugar-protein composition used. While similar concentrations were detected in samples processed at 140°C and 180°C, samples processed at 160°C showed increases from 25% (milk protein/glucose) up to 100% (whey protein/glucose, milk protein/glucose syrup). When looking on the formation of 2,4-decadienal exclusively, one may recommend barrel temperatures of 140°C or 180°C since both resulted in lowest concentrations. It is to consider that flavor formation is a complex system with several variables. While low temperatures may inhibit generating important flavor compounds, high temperature may lead to the formation of different compounds as stated by *Pfannhauser* [71]. He found that 2,4-decadienal is reactive at high temperatures forming benzaldehyde and phenylacetaldehyde as further thermal reaction products. This could be a possible explanation for the decreasing 2,4-decadienal concentration at 180°C barrel temperature.

What was found is that different concentrations at different barrel temperatures cannot be attributed to the examined system parameters. Evaluation showed that elevated tem-

peratures led to consistently decreasing die pressures as well as consistently increasing die temperatures SME values. Only milk protein/glucose syrup samples showed slightly higher SME values at 160°C compared to those found at 140°C and 180°C.

Within this study it was found that 2,4-decadienal formation can be strategically influenced by protein, sugar, and temperature. However, the impact of the detected concentrations on sensory characteristics has to be examined before a final recommendation regarding optimized process parameters is possible.

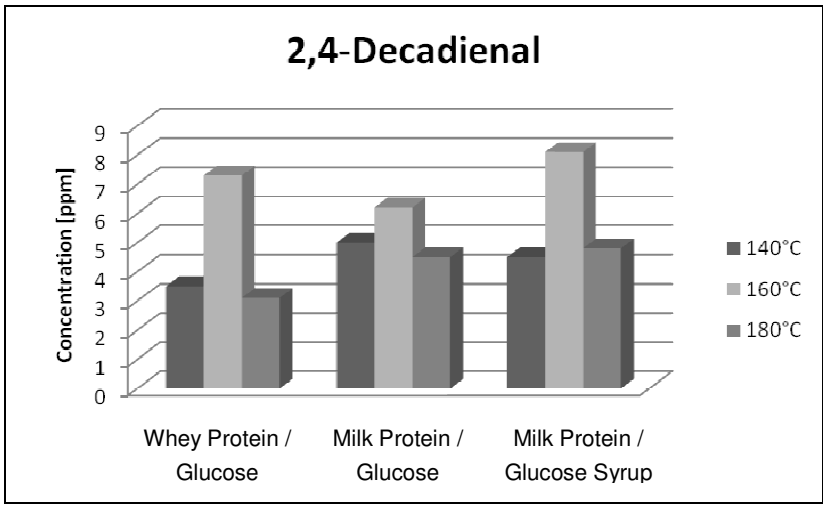


Figure 39: Influence of barrel temperature on 2,4-decadienal formation at constant protein (2%) and sugar (4%) contents.

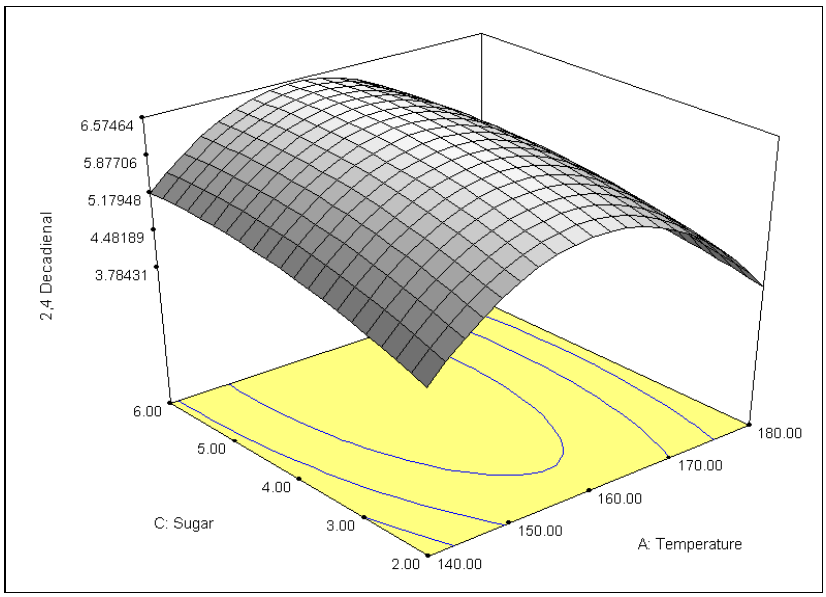


Figure 40: Influence of temperature and glucose contents on 2,4-decadienal formation at a constant milk protein content (2%).

4.2.4 Influences on Acid Formation

Individually seen, organic acids such as butyric acid, pentanoic acid, and hexanoic acid provide powerful, fatty-rancid, and sour odors. Due to interactions with other flavor compounds, acids may contribute to a balanced flavor impression. Within this study, the general influence of sugar, protein, and temperature on acid formation should be examined, regardless of their sensory properties.

Evaluation via DesignExpert® showed a significant influence of protein on the formation of butyric acid and pentanoic acid in the milk protein/glucose system only. However, a closer look should help examining basic trends. **Figure 41** and **Figure 42** show the influence of different sugar and protein concentrations and compositions on the formation of all three examined acids. When using glucose syrup, similar curve progressions at changed sugar concentrations were obtained for all acids. After an intense increase until 4%, a rapid decrease appeared at 6 and 8% sugar. However, butyric acid was differently influenced by changed glucose contents compared to pentanoic acid and hexanoic acid. The latter showed decreasing concentrations from 0 to 4%, followed by increases, while butyric acid started with increasing concentrations and lower amounts at 6 and 8%.

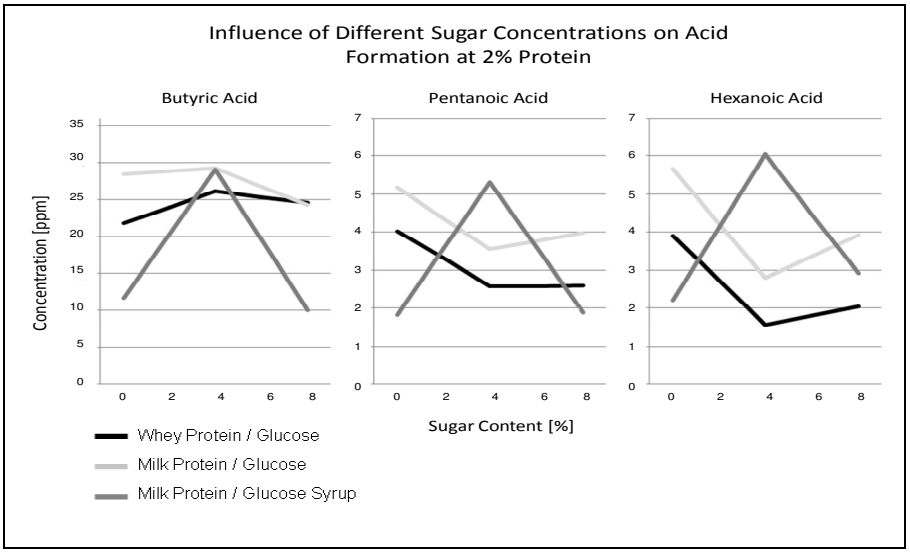


Figure 41: Concentration of butyric acid, pentanoic acid, and hexanoic acid, depending on the amount of sugar at constant protein contents (2%), processed at 160°C.

Evaluation of acid formation at changeable protein concentration showed partly similar results. Again, glucose syrup containing samples came along with a distinct increase until 2% protein, followed by an intense drop at 2.6%. Only hexanoic acid showed a different curve progression.

In contrast to changed sugar concentrations, glucose containing designs showed oppositional curve progressions when raising the protein content. On the one hand, an increase in the milk protein content from 0 to 1.3% resulted in increasing acid concentrations, followed by more or less unchanged concentrations at 2 and 2.6%. On the other hand, increasing whey protein contents resulted in decreasing acid concentrations.

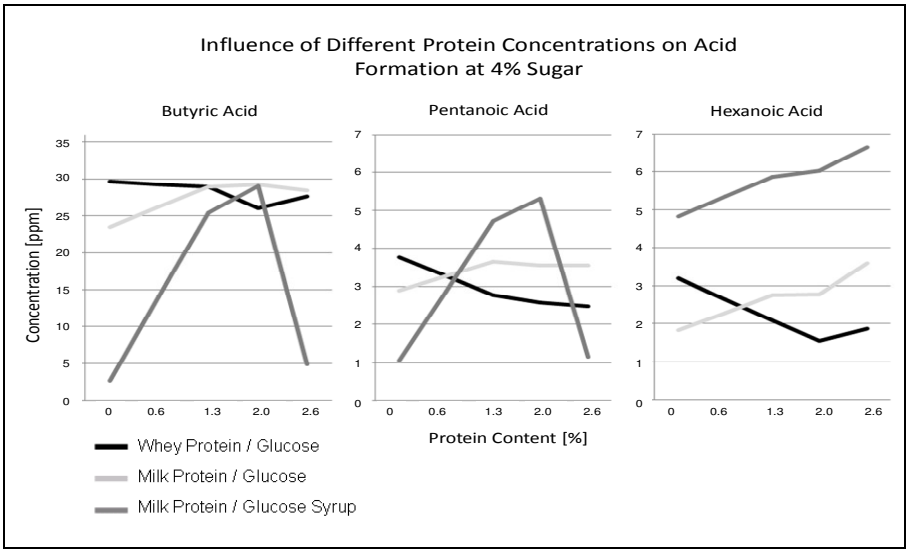


Figure 42: Concentration of butyric acid, pentanoic acid, and hexanoic acid, depending on the amount of protein at constant sugar contents (4%), processed at 160°C.

Obvious similarities in acid concentrations have been found in designs using glucose as sugar source, regardless of the kind of protein. Explicit differences became visible when using glucose syrup. Thus, sugar was found to be an influential parameter for acid formation, whereas protein was not. Slightly different increases and decreases in glucose containing samples depending on the protein source are explicable. Again, it is to taken into account that the source of milk protein was milk powder, containing 60% milk sugar. Hence, detected differences confirm the observed influence of sugar on acid formation.

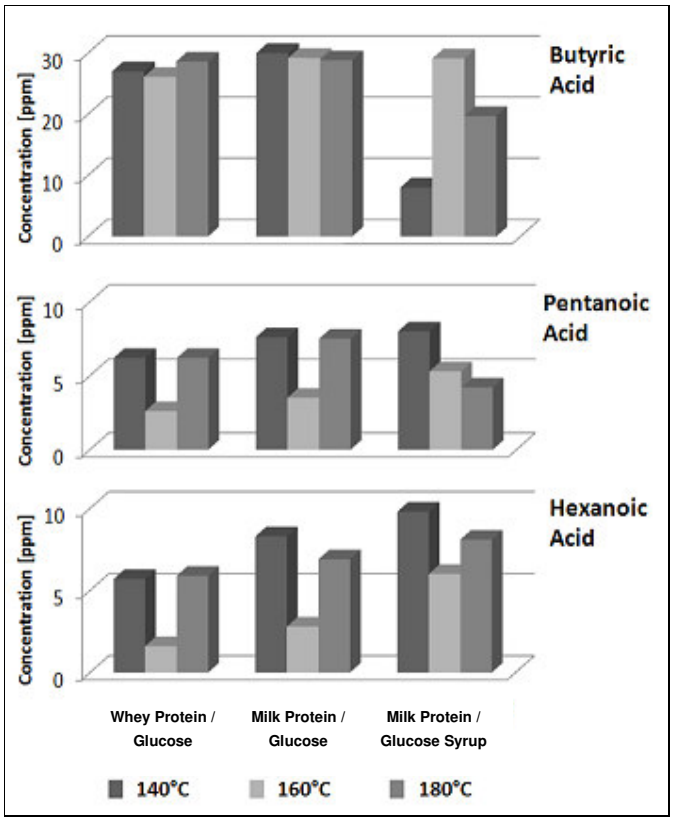


Figure 43: Influence of barrel temperature on the formation of butyric acid, pentanoic acid, and hexanoic acid at constant protein (2%) and sugar (4%) contents.

The influence of temperature on acid formation for all designs is shown in **Figure 43**. Both glucose designs showed comparable results while the results for butyric acid and pentanoic acid from samples containing glucose syrup differed. An explanation for the results is hard to find. The detected minimum concentrations at 160°C barrel temperature cannot be attributed to any of the examined systems parameter. As mentioned earlier, die temperature and SME showed consistently increasing values at increasing barrel temperatures while the die pressure consistently decreased. The same applies to the residence time. Even though the screw speed and the total mass flow remained unchanged, it can be slightly influenced by temperature. As found by *Bindzus* [116], increasing temperatures lead to decreasing residence times due to lower viscosities and faster die flows.

Since the influence of measurable system parameters could be ruled out, different chemical reaction at different temperatures are most likely. A minimum acid concentration at 160°C barrel temperature might be possible due to a favored formation of at least one chemical compound at 160° only.

While the chemical pathway of butyric acid and pentanoic acid is unsettled, the formation of hexanoic acid can be assumed by the cleavage of 2,4-decadienal into hexanal

and hexanoic acid. This theory might be confirmed by comparing the obtained results with those for 2,4-decadienal. While hexanoic acid showed a minimal concentration at 160°C, 2,4-decadienal showed its maximum at the same temperature. It should be emphasized that this is only one possible explanation. It does not explain the similarity of pentanoic acid since it cannot have been synthesized from 2,4-decadienal.

4.2.5 Influences on Maltol Formation

As indicated earlier, the determination of maltol concentration was made with respect to its importance as an aroma active compound. Results are precarious since no stable isotope dilution analysis was carried out for maltol. All values are based on comparisons with those for the internal standard 1-octanol. Thus, no real concentrations are displayed and rough tendencies could be derived only.

As can be seen in **Table 17** to **Table 19** and in , the influences of all three changeable parameters strongly depended on the design. While temperature, protein, and sugar showed significant influences on maltol formation in whey protein/glucose samples, no significance was found for samples containing milk protein and glucose syrup. The influence of different sugars and protein as well as their amounts clearly becomes visible in **Figure 44**, **Figure 45**, and **Figure 46**.

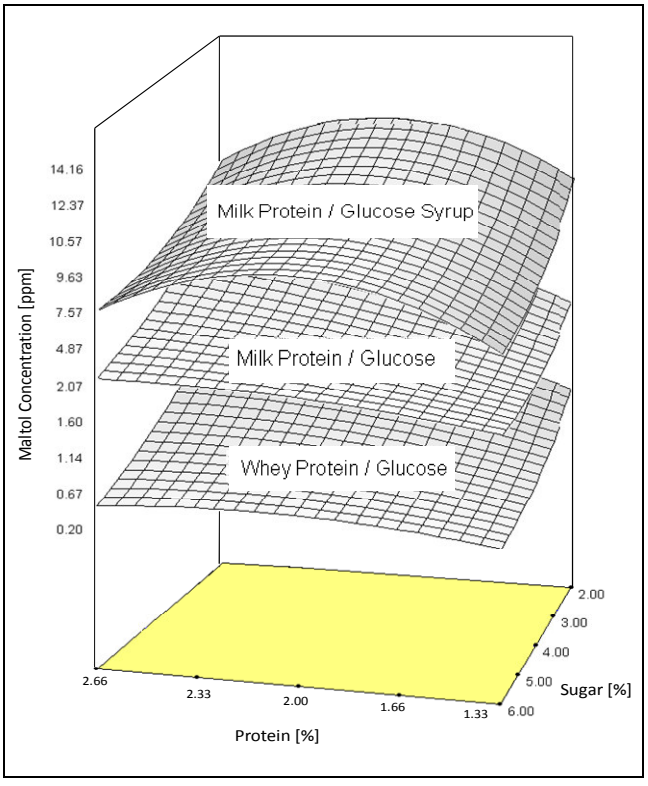


Figure 44: Influence of different protein-sugar compositions on maltol formation at 160°C barrel temperature.

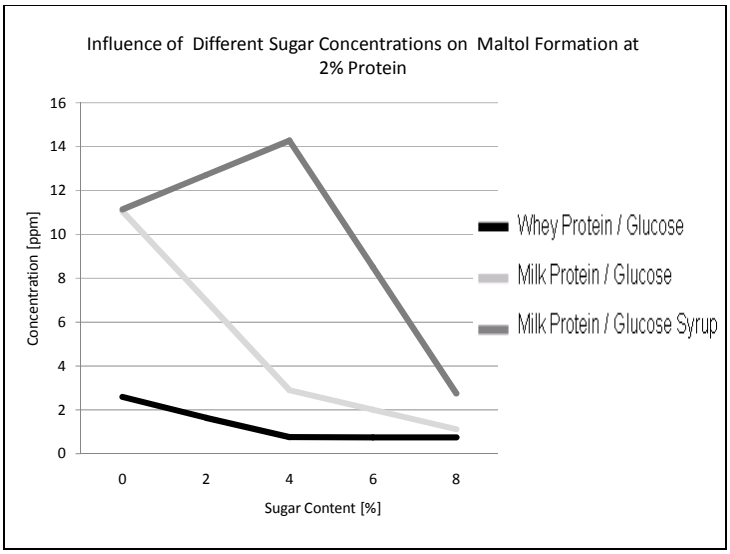


Figure 45: Concentration of maltol, depending on the amount of sugar at constant protein contents (2%), processed at 160°C.

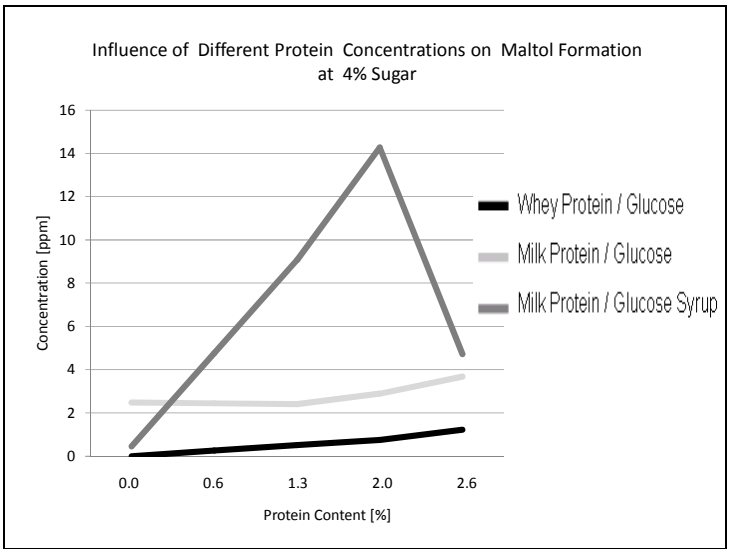


Figure 46: Concentration of maltol, depending on the amount of protein at constant sugar contents (4%), processed at 160°C.

Both designs using milk protein started with a five times higher amount of maltol at 0% sugar compared to the design using whey protein. The reason could be found in the nature of the protein source milk powder, naturally containing milk sugar. Milk sugar as well as glucose syrup may act as maltol precursors and thereby support maltol formation, whereas glucose cannot. Since protein was found to promote maltol formation, an increased glucose concentration may counteract this by forming different compounds, leading to decreased maltol concentrations at elevated glucose contents. In contrast, the use of up to 6% glucose syrup at 2% protein led to an increasing maltol formation. As

mentioned above, glucose syrup contains sugars which may act as maltol precursors. At glucose syrup concentrations higher than 6%, an excess of sugar molecules faces a lack of protein. Increasing amounts of glucose, which is a component of glucose syrup as well, reacts easier with protein forming different flavor compounds leading to decreasing maltol concentrations.

The distinct drop of maltol concentration at 2.6% milk protein and 4% glucose syrup, as illustrated in **Figure 46**, was unexpected. A lack of necessary sugar at elevated protein contents might be a reason but remains unlikely since the decrease was found to be too distinct. This is in contrast to designs using glucose. Irrespective of the protein used, a slight and consistent increase was found. Thereby, milk protein/glucose samples consistently showed 1.9 to 2.4 ppm higher concentrations than whey protein/glucose samples. Again, this was found to be due to milk sugar, partly acting as maltol precursor.

Summarizing it can be stated that sugar was found to be the most influential parameter on maltol formation. This applies for its concentration as well as for its kind. The use of different proteins resulted in similar behaviors regarding maltol formation when using the same sugar. Within this study, differences in the total amount of maltol is most likely due to the protein source.

The influence of temperature on maltol formation for all three designs is depicted in **Figure 47**. Again, glucose containing designs showed similarities while the use of glucose syrup did not. On the contrary, it resulted in oppositional effects.

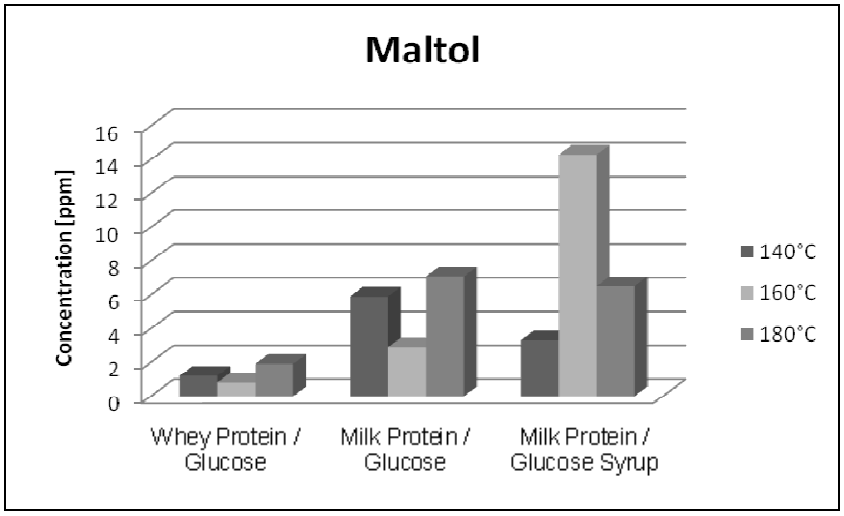


Figure 47: Influence of barrel temperature on the formation of maltol at constant protein (2%) and sugar (4%) contents.

Glucose samples are characterized by a minimum maltol concentration at 160°C and values about twice as high at 140°C and 180°C. Only the total amounts differed, depending on the protein. The use of milk protein resulted in up to 5 times higher concen-

trations compared to samples containing whey protein. As mentioned previously, this is due to additional milk sugar from the protein source milk powder. An oppositional effect was found for glucose syrup. At 160°C, a maximum concentration was found.

Neither the minimum concentration at 160°C in glucose samples nor the maximum concentration at 160°C in glucose syrup samples can be explained. Furthermore, no coherences have been found between the obtained minimum or rather maximum concentrations and any system parameter such as SME, die pressure, or die temperature.

Looking at maltol as a desired flavor compound, the use of glucose should be minimized since it limits the maltol formation. The use of alternative sugar might be considered since investigations showed a positive influence of milk sugar as well as glucose syrup. Additionally it was found that the formation of maltol rests upon interactions between sugars and proteins. Thereby, already small amounts of sugar showed high potentials to form maltol in the presence of protein whereas high amounts led to decreasing concentration explainable by the formation of different flavor compounds, favored at elevated sugar concentrations

4.3 Influence of Process Parameters on System Parameters

Beside the direct influence of the changeable process parameters on flavor development, the effect on their derived system parameters was to be examined. Barrel temperature, sugar and protein in different compositions were analyzed regarding their influence on specific system parameters. In this case, die temperature, representing the final product temperature, die pressure, and specific mechanical energy (SME) were measured for designs using milk protein and glucose syrup as well as whey protein and glucose. They were analyzed for the defined experimental space by statistical evaluation, based on polynomial regression. **Table 20** and **Table 21** show the calculated regression coefficients, valid for the analyzed experimental designs, using milk protein and glucose or whey protein and glucose syrup. For the evaluation of functional relations, only terms showing impact significances >95% (bold values) were used. Additionally, interactions of all three system parameters were analyzed. The model chosen for evaluation depended on the recommendation given by the statistic software and guaranteed the most appropriate fit. The results are shown in **Table 22**. Thereby, SME was found to be significantly influenced by increasing die pressure whereas no further significant interactions could be found.

Table 20: Regression coefficients and coefficients of determination for the influence of process parameters on system parameters using milk protein and glucose syrup

Source	Factor	Regression Coefficient		
		Die Temperature	Die Pressure	SME
Model	-	**+174.28**[2)]	**+4.04**[1)]	**+124.12**[1)]
Linear	Barrel Temperature	**+3.50**[3)]	**-2.14**[2)]	**+4.94**[1)]
	Protein	**-1.30**[1)]	-0.83	+2.13
	Sugar	**-1.80**[2)]	+0.53	**-8.31**[2)]
Quadratic	Barrel Temperature	+2.09	+0.98	**-9.50**[1)]
	Protein	-0.91	+0.16	-0.25
	Sugar	+0.59	+0.38	-0.55
Interactive	Barrel Temp.-Protein	+0.25	+0.82	-0.30
	Barrel Temp.-Sugar	**-1.25**[1)]	-1.03	-0.90
	Protein-Sugar	0.000	-0.23	+1.78
Coefficient of Determination		0.9416	0.8731	0.8681

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)

Table 21: Regression coefficients and coefficients of determination for the influence of process parameters on system parameters using whey protein and glucose

Source	Factor	Regression Coefficient		
		Die Temperature	Die Pressure	SME
Model	-	**+184.69**[2)]	**+6.41**[2)]	+120.07
Linear	Barrel Temperature	**+4.30**[3)]	**-1.92**[3)]	**+4.28**[1)]
	Protein	0.000	**-2.04**[3)]	+2.16
	Sugar	**-2.10**[2]	+0.65	**-6.33**[2)]
Quadratic	Barrel Temperature	-1.53	**+2.21**[2)]	+0.39
	Protein	+0.97	-0.41	-0.70
	Sugar	-1.53	+0.036	-3.66
Interactive	Barrel Temp.-Protein	-0.38	-0.068	+3.19
	Barrel Temp.-Sugar	+0.13	**-1.29**[1)]	-1.84
	Protein-Sugar	-0.37	-0.23	-2.66
Coefficient of Determination		0.9640	0.8734	0.8387

Bold values indicate significance ([1] P>95%, [2] P>99%, [3] P>99.9%)

Table 22: Statistical correlation of system parameters

	Product Temperature	**Product Pressure**	**SME**
	Model: linear	Model: quadratic	Model: quadratic
	R^2=0.5658	R^2=0.7236	R^2=0.736
Product Temperature		-24.66	+33.24
Product Pressure	-5.53		**+53.88[1)]**
SME	+5.56	+0.19	

Bold values indicate significance ([1] P>95%, [2] P>99%, [3] P>99.9%)

As expected, the barrel temperature was the most important parameter influencing all examined system parameters with high degrees of statistical significance. Beside an increase in die temperatures, the increase in barrel temperatures led to a significant decrease in die pressure, due to lower viscosities at higher temperatures.

Furthermore, analysis showed a significant increase in SME when temperatures were elevated. This was astonishing and unexpected. In general, increasing temperatures are accompanied by a simultaneous drop in die pressure due to lower viscosities. As a consequence, decreasing SME values were expected. A possible explanation might be an interaction of sugar and temperature.

In addition to the barrel temperature, increasing sugar contents significantly influenced the die temperature and the SME. Both effects were found to be independent from the kind of sugar. These results are expected. At higher extrusion temperatures more sugar tends to melt, resulting in a decrease of the viscosity, the prime reason for decreasing die temperature and SME. Additionally, the melting of the sugar may interfere with the melting of the starch and thereby may also affect SME. The complex influence of sugar on system parameters becomes visible when looking at changes in die pressure. No significant influence was diagnosed. This lies in the nature of the sugar. The higher the temperature the more liquid it becomes. At 140°C, increasing sugar contents raise the die pressure while the already lower die pressure at 180°C remains more or less constant, as illustrated in **Figure 48**. It is to be added that pressure data as they are given in this thesis do not represent realistic values. It was found that the used pressure sensor was most likely not or only insufficiently gauged. Therefore pressure values have to be seen relatively to each other only.

Differences among the analyzed designs were found regarding the influence of protein. While whey protein showed no influence on the die temperature but an expected, significant drop in die pressure, the milk protein seemed to influence the die temperature in a significantly negative way, but not the die pressure. This is due to the protein source, which was non-fat dried milk powder. Beside 33.4% milk protein it contained 54% milk sugar, responsible for the found oppositional effects. This explains the results and confirms the influence of sugar as stated above.

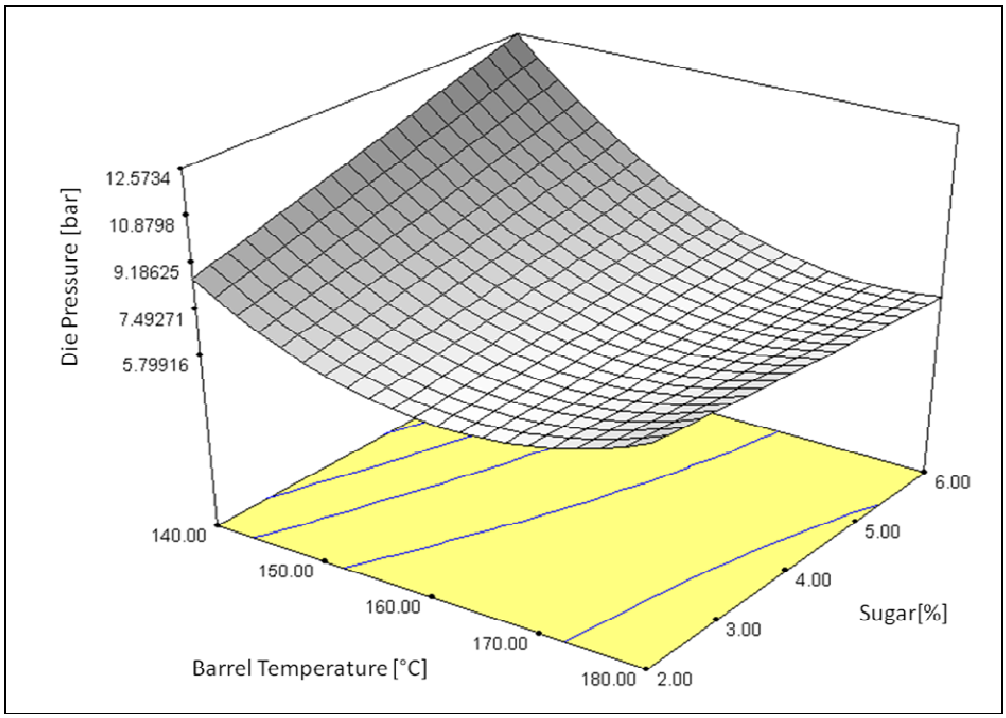


Figure 48: Changes in die pressure depending on glucose content and barrel temperature at a constant whey protein content of 2%.

4.4 Influence of System Parameters on Product Parameters

In view of a possible process controlling and optimization, system parameters were checked for coherences between their variations and the formation of important flavor compounds. Therefore, methylpyrazine, 2,5-dimethylpyrazine, furanmethanol, 2,4-decadienal, and hexanoic acid were analyzed regarding their interactions with the system parameters die temperature, die pressure, and SME. After evaluating the most appropriate fit, the linear model was chosen. This does not allow drawing any conclusions about interactions between system parameters and does not show quadratic coherences. Nevertheless, it is the model of choice since it is significant for most of the chosen product parameters in contrast to the quadratic model as it was used previously.

Table 23: Regression coefficients and coefficients of determination for the influence of system parameters on product parameters using whey protein and glucose

Source	Factor	Methylpyrazine	2,5-Dimethylpyrazine	Furanmethanol	2,4-Decadienal	Hexanoic Acid
Model	-	**+0.47**[1)]	**+0.26**[3)]	**+2.44**[2)]	-0.16	+0.97
Linear	A	+0.75	+0.078	+0.77	+3.65	+2.30
	B	-0.13	-0.0075	-0.078	+0.36	+0.07
	C	+0.31	**+0.29**[2)]	**+2.15**[2)]	-1.53	+0.41
Coefficient of Determination		0.4693	0.7691	0.6620	0.1338	0.2492

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)
A=die temperature, B=die pressure, C=SME

Table 24: Regression coefficients and coefficients of determination for the influence of system parameters on product parameters using milk protein and glucose syrup

Source	Factor	Methylpyrazine	2,5-Dimethylpyrazine	Furanmethanol	2,4-Decadienal	Hexanoic Acid
Model	-	**+0.27**[3)]	**+0.024**[3)]	**+10.00**[2)]	+6.61	**+-0.29**[2)]
Linear	A	+0.19	**+0.085**[2)]	+7.43	-1.07	+65.50
	B	-0.025	-0.0049	-0.60	+0.14	+44.72
	C	**+0.25**[2)]	**+0.048**[3)]	**+7.98**[1)]	+1.35	**-47.76**[2)]
Coefficient of Determination		0.7725	0.8618	0.6300	0.1153	0.6406

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)
A=die temperature, B=die pressure, C=SME

As can be seen in **Table 23** and **Table 24**, there is only little statistical significance to be found between system parameters and product parameters. Only SME showed significant influences on 2,5-dimethylpyrazine and furanmethanol in both designs. Additionally, methylpyrazine and hexanoic acid were significantly influenced by SME and 2,5-dimethylpyrazine by the die temperature when using milk protein and glucose syrup. The influence of SME and die temperature on 2,5-dimethylpyrazine formation is shown in **Figure 49** as an example for the design using milk protein and glucose syrup.

The determined lack of statistical significance did not come unexpectedly. When analyzing the influence of process parameters on flavor development, only pyrazines and furanmethanol were found to be influenced by temperature. Both protein and sugar showed more distinct effects on flavor formation. Furthermore, it must be taken into account that temperatures, measured at the die, showed smaller ranges compared to the directly controlled barrel temperature. While the barrel temperature varied from 140°C to 180°C over a range of 40°C, the measured die temperature was between 171°C to

183°C for milk protein/glucose and 176°C to 189°C for whey protein/glucose. The resulting range of only 12°C or rather 13°C was too small to allow high degrees of statistical certainty. The die pressure was influenced by too many factors to be an appropriate influential parameter itself.

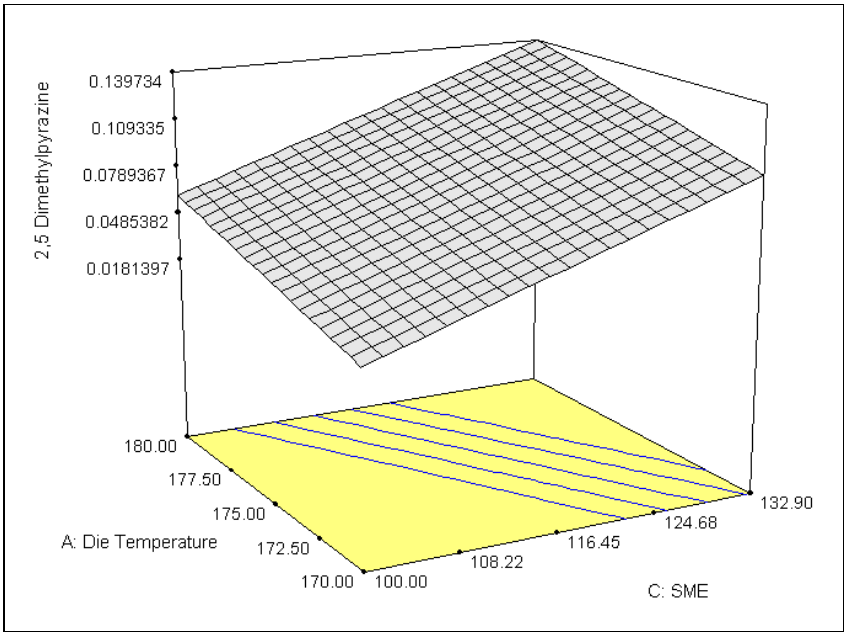


Figure 49: Influence of SME and die temperature on 2,5-dimethylpyrazine formation in a milk protein/glucose syrup design at medium die pressure (approx. 6 bar).

However, what remains is the SME. Finding it positively influencing the formation of pyrazines as well as furanmethanol is of importance. A higher input of mechanical energy can be realized by increasing shear forces. The latter can be obtained by modified screw configurations. This knowledge offers a possibility for future process optimization.

4.5 Influence of Single Amino Acids as Potential Flavor Precursor

Amino acids are important flavor precursor since they provide much more free amino groups than complex proteins. The concentrations of selected flavor compounds, depending on the added amino acids and their concentrations are listed in **Table 25**. In preliminary extrusion trials, starch, whey protein, and glucose were processed without additional amino acids under similar conditions of temperature, moisture, and screw speed and at the same starch-protein-sugar composition. The resulting concentrations of flavor compounds are given as reference values.

Table 25: Flavor compound concentration depending on amino acids

Trial	Amino Acid [ppm]	Methyl-pyrazine	2,5 Dimethyl-pyrazine	Furan-methanol	Maltol	2,4-Decadienal	Butyric Acid	Pentanoic Acid	Hexanoic Acid
Valine	250	2.06	0.38	3.08	0.61	4.4	8.94	0.97	1.66
	1000	2.19	0.51	3.05	0.88	3.6	10.20	1.10	1.85
Proline	250	1.97	0.48	3.37	0.95	4.8	9.37	1.19	2.03
	1000	2.33	0.59	3.90	1.41	4.4	10.32	1.46	1.91
Leucine	250	2.76	0.61	3.76	0.98	7.3	30.09	2.62	2.27
	1000	2.97	0.66	3.68	0.58	6.8	26.64	1.92	1.30
Cysteine	250	1.47	0.54	3.76	0.99	5.4	23.05	3.92	3.60
	1000	2.01	0.68	4.90	1.16	2.63	26.72	3.81	3.48
Reference	0	2.08	0.32	2.91	0.76	7.3	26.05	2.58	1.56

All extrudates processed at 160°C, 4% glucose, 2% whey protein; flavor compound concentrations in ppm

The influence of different amino acids on the formation of examined flavor compounds was found to be variable, making an overall evaluation impossible. Differences were found between the amino acids, their concentration and their impact on different compounds.

Pyrazines were affected differently by amino acids. As can be seen in **Figure 50**, the most obvious influence was found for 2,5-dimethylpyrazine. Already at 250 ppm added amino acids, its concentration showed increases from about 20% using valine, up to 100% using leucine. At 1000 ppm, the concentration of 2,5-dimethypyrazine at least doubled for proline, leucine, and cysteine and even valine led to an increase of about 65%. In contrast, the influence on methylpyrazine formation was quite low. Whereas no changes at all could be found using valine and proline, the addition of 250 ppm cystein resulted in a 30% lower concentration compared to the reference sample. Only leucine showed an increase of about 30% at 250 ppm and nearly 50% at 1000 ppm. All in all, leucine was the most influential amino acid regarding the formation of both dimethylpyrazine and 2,5-dimethylpyrazine.

The general increase in pyrazines due to added amino acids did not come unexpectedly. Pyrazines are important nitrogen-containing flavor compounds which are generally formed in the reaction of an amino group with a carbonyl group in the Maillard browning reaction. The results are in line with examinations made by *Hwang* et al. and *Riha* et al., who stated that the formation of most pyrazines is positively influenced by amino acids, regardless of their type [56, 49]. This is partly confirmed by *Farouk* et al. who found pyrazine contents increased with the addition of all amino acids, except cysteine which enhanced the formation of sulfur compounds instead of pyrazines [62].

However, due to the presence of both a sulfur and nitrogen atom on cysteine one may expect these different classes of compounds to be produced. Within the chemical reactions, resulting in the formation of flavor compounds, sulfur and nitrogen do not have to compete for necessary carbonyl groups. The latter are available in excess due to added glucose and are responsible for finding 2,5-dimethylpyrazine formation positively influenced by added cystein.

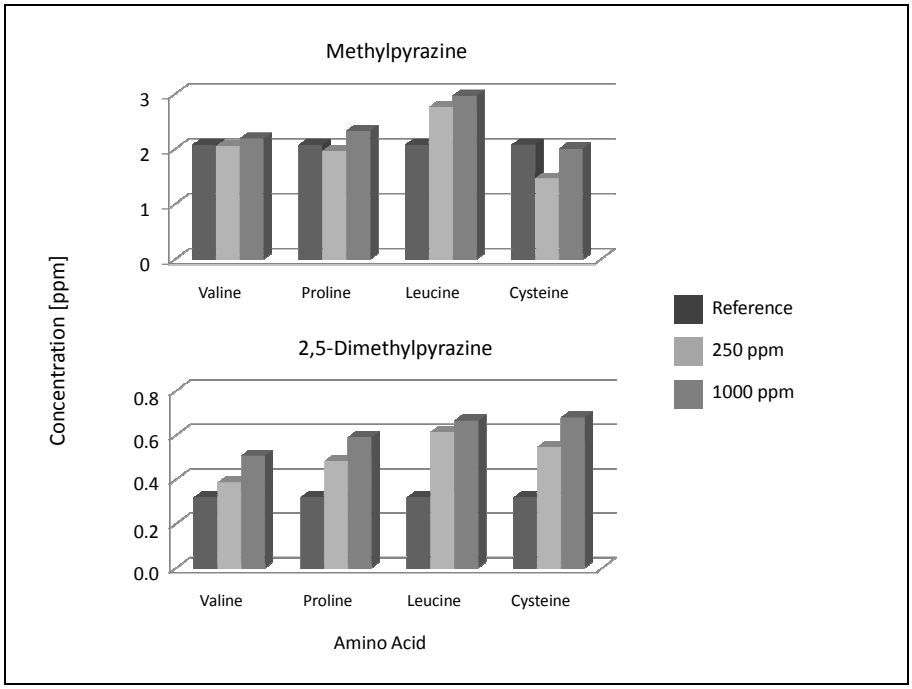


Figure 50: Differences in methylpyrazine and 2,5-dimethylpyrazine formation, depending on the kind and concentration of added amino acids.

The influence on acid formation strongly depends on the examined acid and the used amino acid. While the use of valine and proline led to a significantly lower concentration of butyric and pentanoic acid compared to the reference sample, leucine and cysteine samples showed similar or slightly increased concentrations. The concentration of hexanoic acid rose with all amino acids but most slightly using valine, proline, and leucine with an increase from 0% to 30%. Only the use of cysteine resulted in up to 130% higher concentrations. Leucine was found to be the only amino acid with distinctly lower amounts of all three examined acids at 1000 ppm compared to 250 ppm. With regard to the total acid concentration, changes caused by added amino acids are negligible. This and their minor role as flavor compounds might be the reason for the fact that they have never been mentioned in reviewed literature examining the influence of amino acids.

An only slight but consistent effect was found on furanmethanol formation. All amino acids led to an increase of about 5% to 30% except cystein at 1000 ppm, resulting in an

increase of about 70%. Changes in furanmethanol concentrations due to added single amino acids have not been reported yet. In general, the resulting increases seem to be too low to significantly influence the final flavor impression.

The influence on maltol formation is depicted in **Figure 51**. Maltol is an important flavor compound that is found in white bread crust and is well known as a flavor enhancer. *Farouk* et al. found maltol to be a major volatile product in extrudates containing glucose and amino acids and underlined its high concentrations while using leucine at concentrations of 0.5% and 2% [62].

This is in contrast to results found within this study. As for all examined compounds, except pyrazines, the addition of leucine resulted in a slightly increasing concentration at 250 ppm, followed by a decrease at 1000 ppm or rather 0.1%. In how far a further elevation of the lysine concentration up to a level as it was used by *Farouk* et al. would influence the maltol formation cannot be answered. A consistent increase, resulting in maltol levels up to 100% higher than detected in the reference sample, was found for proline and cysteine. Both took no part within other studies examining the influence of single amino acids on the formation of maltol. As a conclusion of this study, the additional use of proline or cysteine is preferable over the use of valine or leucine.

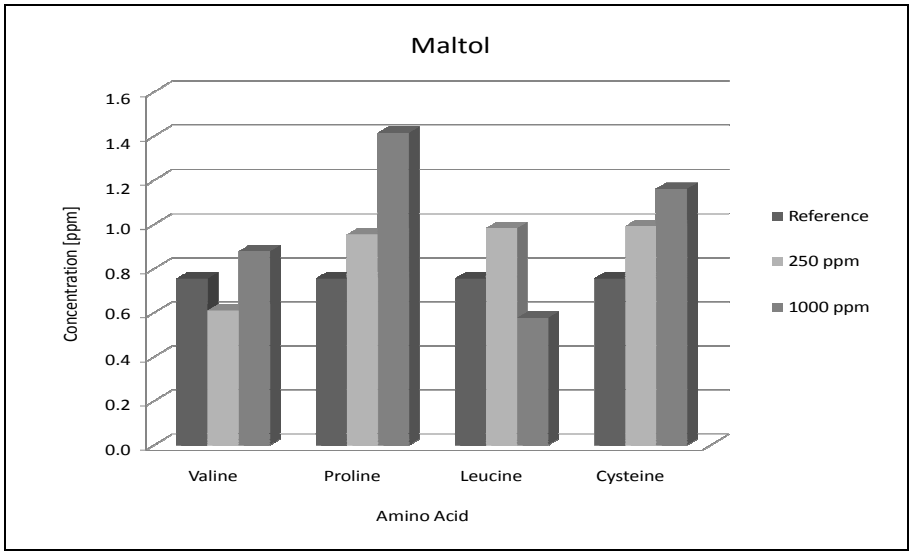


Figure 51: Differences in maltol formation, depending on the kind and concentration of added amino acids.

Results found for 2,4-decadienal formation are comprehensible and were expected. Amino acids have the same effect on the generation of 2,4-decadienal as protein. Both provide amino groups, necessary for the formation of numerous chemical compounds. These formations often require sugar as a source of reactive carbonyl groups. As mentioned in chapter 4.2.3, this leads to a reduced availability of sugar for oxidation processes, important for the formation of 2,4-decadienal. The effect of different amino acids

is shown in **Figure 52**. Beside leucine, the addition of amino acids resulted in 2,4-decadienal reductions of 25% to 40% at 250 ppm and 40% to 65% at 1000 ppm.

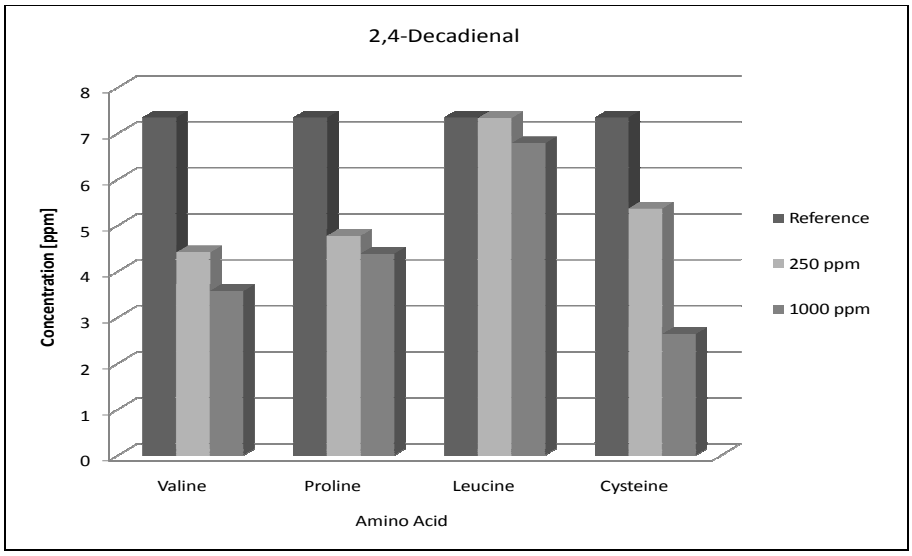


Figure 52: Differences in 2,4-decadienal formation, depending on the kind and concentration of added amino acids.

It was found that by addition of selected single amino acids the formation of desired flavor compounds can be strategically influenced. The results are in line with reviewed literature, confirming that the generation of flavor components depends on the amino acid used. For example, leucine exclusively enhanced the formation of methylpyrazine significantly. Simultaneously, it was the only of the examined amino acids that did not lead to a distinct reduction of 2,4-decadienal, a potent off-flavor at higher concentrations.

It was found by *Hofmann* and *Schieberle* [50] and confirmed within this study that the addition of cysteine favors the formation of volatiles such as 2,5-dimethylpyrazine, responsible for desired roasted, popcorn-like flavor impressions. But it remains to be considered that cysteine may cause unpleasant sulfur odor notes as it was found by *Wright* et al. [58].

Because of these specific attributes, the application of single amino acids remains complicated and should not be realized without complex previous sensory studies.

4.6 Color Measurement

Color plays an important role in the general acceptance of food by the consumer. Color measurement was carried out to determine the influence of sugar, protein, and temperature on product lightness. The complete set of data is enclosed in the appendix, **Table A 4**. All lightness values are given as relative lightness L, (L=100-L*).

Table 26: Regression coefficients and coefficients of determination for the influence of process parameters on product lightness L

Source	Factor	Regression Coefficient		
		L Milk Protein/ Glucose Syrup	L Milk Protein/Glucose	L Whey Protein/Glucose
Model	-	**+29.31**[1)]	**+31.21**[2)]	**+31.72**[3)]
Linear	Barrel Temperature	+0.87	**-1.50**[2)]	**+1.34**[2)]
	Protein	**+2.29**[2)]	**+1.46**[2)]	**+0.97**[2)]
	Sugar	**+1.28**[1)]	**+2.43**[3)]	**+3.66**[3)]
Quadratic	Barrel Temperature	-2.25	**-1.47**[1)]	**-2.01**[2)]
	Protein	-0.37	+0.65	+1.13
	Sugar	-0.18	-1.16	-1.01
Interactive	Barrel Temp.-Protein	+0.15	-0.26	-0.30
	Barrel Temp.-Sugar	+0.39	-0.08	+0.69
	Protein-Sugar	+0.92	-0.028	+0.27
Coefficient of Determination		0.8797	0.9571	0.9799

Bold values indicate significance ([1)] P>95%, [2)] P>99%, [3)] P>99.9%)

As can be seen in **Table 26**, all parameters showed significant influences. Only the barrel temperature did not show any significance for samples containing milk protein and glucose syrup. In general, a quadratic effect of increasing temperatures on color development was found, resulting in initially increasing darkening followed by decreases. The temperature at which a maximum darkening was detected strongly depended on the sugar-protein composition. For milk protein/glucose it was found to be close to the minimum temperature of 140°C. This resulted in a significant negative influence of an increasing barrel temperature since it ranged from 140°C to 180°C. On the other hand, the most intense color development was found around 170°C for whey protein/glucose samples, resulting in a significant positive influence within the analyzed design space. Summarizing it was found that temperature does influence product darkening. However, the way it influences the color strongly depends on product composition and the chosen

range of temperature. A possible reason for the quadratic effects found is an intensified expansion at high temperature. Air inclusions within the starch-protein-sugar matrix might be responsible for a lighter appearance. Further investigations on resulting product densities are necessary for a conclusive explanation.

Both protein and sugar showed a significant influence on color development. Increasing sugar concentration resulted in increasing L values, synonymic to intensified darkening, due to browning reactions such as caramelization and the Maillard reaction. As can be seen in **Table 26**, this effect is more pronounced when using glucose, indicated by higher regression coefficients. It is additionally visualized in **Figure 53**. While glucose syrup showed only a slight increase from L=25 at 0% sugar to L=31 at 6%, followed by a decrease at 8%, glucose samples reached their maximum at 8% sugar with an approximated lightness value of 36. The initial lightness for designs using glucose depended on the added protein. The use of whey protein resulted in a minimum lightness of 12 at 0% sugar, whereas glucose/milk protein showed an initial lightness of around 21 at 0% sugar. The latter is relatively close to the value for milk protein/glucose syrup.

The reason is evident. As mentioned previously, the source of milk protein is milk powder, containing 54% milk sugar. The ratio of sugar to protein in milk powder is about 5:3. Thus, 2% added milk protein is equivalent to 3.3% sugar, responsible for browning reaction. The ability of glucose to favor browning reactions becomes clear finding similar L values already at 2% added glucose, easily compensating the excess in milk sugar.

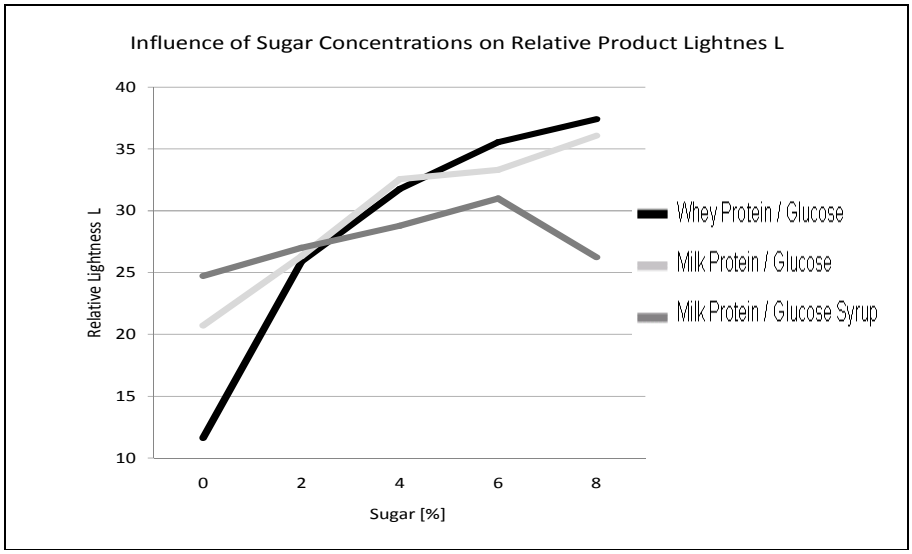


Figure 53: Influence of sugar concentrations on product lightness at 2% protein constantly for samples processed at 160°C, containing different protein and sugar compositions.

As well as sugar, protein significantly influenced product darkening, affected by Maillard reactions. The influence of different proteins and protein contents is shown in

Figure 54. Over all 5 protein levels, glucose containing designs showed nearly identical lightness values. While milk protein/glucose syrup samples showed a similar curve progression, their L values were consistently about 5 points lower. Again, the ability of glucose to affect browning reactions became clearly visible. The influence of the examined proteins was independent of their kind whereas their increasing amounts significantly favored color development.

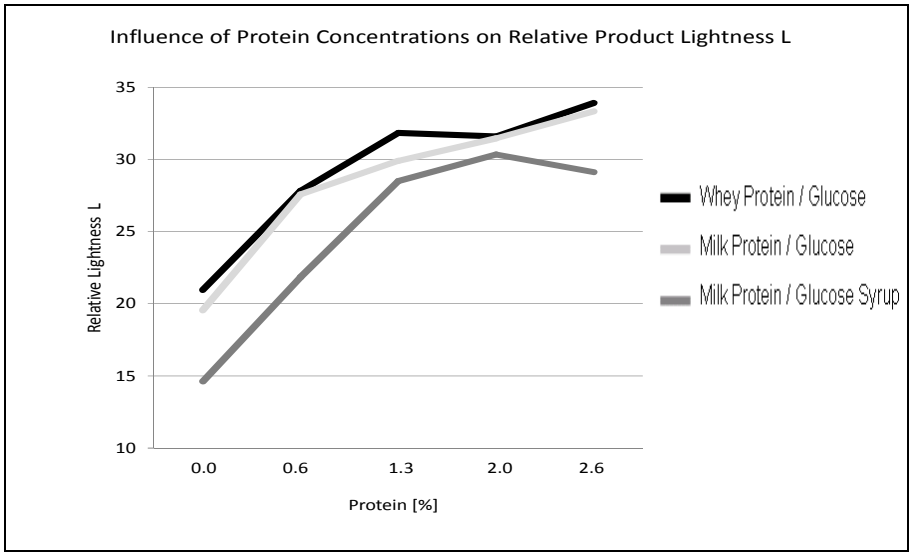


Figure 54: Influence of protein concentrations on product lightness at 4% sugar constantly for samples processed at 160°C, containing different protein and sugar compositions.

For both protein and sugar detected increases were more pronounced from 0 to 1.3% protein or rather 0 to 4% sugar. Only slight increases or even decreases were detected at further elevated protein and sugar contents, leading to the assumption that color development is characterized by an exponential curve progression. This results in the reach of a maximum lightness value that cannot be further increased.

After all, increasing sugar and protein contents were found to positively affect color development. While the used proteins showed no differences in their ability to favor color development, different sugars did. The results are due to intensified Maillard reaction and are in line with findings made by *Köhler* [34] who examined the effect of different sugars on the intensity of color development. He found intensified browning in samples containing the pentose xylose compared to samples containing the hexoses glucose or fructose which is due to its shorter chain length. Glucose syrup consists of a multiple of different complex sugars which do not favor Maillard reaction with such intensity as glucose does. Furthermore, it was found that increasing sugar contents resulted in a more distinct color formation than increasing protein contents. A possible

explanation is the additional formation of colored compounds by caramelization processes.

It was found that elevated temperatures and increasing protein and sugar contents led to significant changes in the concentration of selected flavor compounds and to a significant color development. However, within this study, coherences between color development and the formation of specific flavor compounds could not be found. Again, this is explainable by the fact that the examined flavor compounds are exclusively formed during Maillard type reactions whereas different pathways are responsible for the formation of brown color. At extrusion temperature, added sugar easily caramelizes, resulting in the formation of colored compounds. Using the example of the whey protein/glucose model system, the independency of flavor compound concentration and product lightness is shown in **Figure 55**.

The gained information may help to specifically influence the color of the final product, but the color itself cannot be used as an analytical tool regarding the control of flavor development.

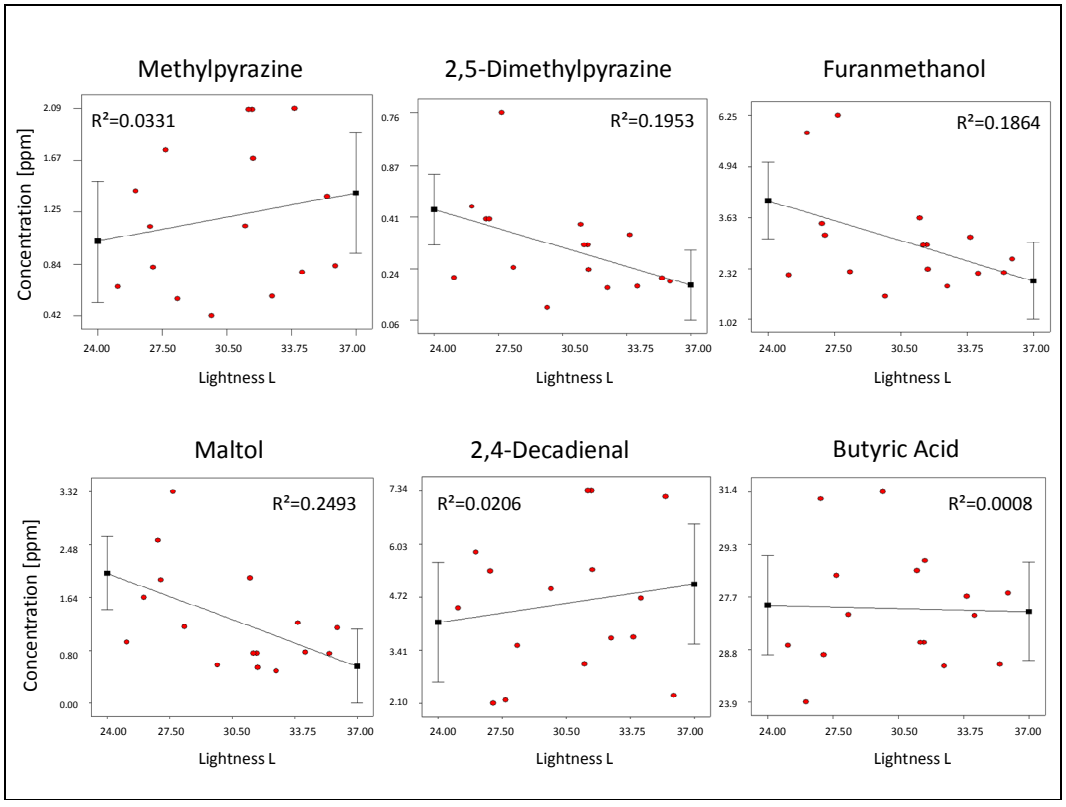


Figure 55: One factor plots, showing the influence of selected flavor compound concentrations on relative product lightness L (0=white, 100=black) using the example of the whey protein/glucose model system.

Appendix

Table A 1: Flavor compounds and their concentrations as detected by headspace sampling in samples containing milk protein and glucose syrup

Protein Source / Sugar	Dryed non-fat Milk Powder/Corn Syrup															
Temperature [°C]	140	140	140	140	140	180	180	180	180	180	160	160	160	160	160	160
Protein [%]	1.3	1.3	2	2.6	2.6	1.3	1.3	2	2.6	2.6	2	2	2	1.3	2	2.6
Sugar [%]	2	6	4	2	6	2	6	4	2	6	2	4	6	4	4	4
1-butanol	0	0	0	0	0	2	1.2	5.6	8.1	5.1	0	0	0	0	1.8	0
1-hydroxy-2-propanone	114	62.9	73.4	59.8	53.3	85.8	61.1	70.8	62.8	71.6	42.6	76.6	72.8	32.8	57.6	69.1
2,3-pentanedione	5.5	4.7	6.7	9.7	5.5	9	4.8	7.5	8.2	6.4	4.4	5.6	6.7	5.5	8.3	7.4
3-hydroxy-2-butanone	8.5	4.5	5.2	4.6	3.4	6.7	5.1	5.8	5.6	6.4	4.3	8.1	6.4	5.6	6.7	5
pyrazine	2	0	0.8	1	0	3.9	0	2.4	2.4	2.8	3.3	0	0	0	3.8	2.9
pentanol	4.1	3.4	4.8	7.9	4.2	4.7	2.9	2.7	2	1.2	1.7	1.8	2.2	0.7	2.9	3.2
butyric acid	2.2	0	0	0	0	1.3	0	0	0	0	0	0	0	0	0	0
hexanal	61.6	64.8	63.3	110	51.3	91.9	62.7	63.8	61.7	42	41	66.4	44.8	43.8	64.7	64.5
dihydro-2-methyl-3(2H)-furanone	5.5	1.8	2.9	2.6	1.5	6.2	2.9	4.9	5.4	4.5	3.1	4.8	2.8	2.6	2.4	2.7
methylpyrazine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4-hydroxy-4-methyl-2-pentanone	17	7.2	8.7	6.8	3.1	8.8	20.5	52.6	70.1	91.7	7.1	9.4	5.4	4.6	7.3	6.2
furancarboxaldehyde	5.9	3.2	4.5	4.9	4	9.4	6.3	9.9	11.7	10.4	6.9	9.3	6	4.5	7.1	6.6
furanmethanol	58.8	27.8	32.5	26.4	17.3	54.6	16.6	27.1	23.7	16.1	18.9	22.9	13.7	4.9	10.8	11.8
4-heptanone	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218
3-methyl-2-butanone	3.9	3	3.7	5.1	2.9	4.1	3.6	3.4	2.8	3.2	2.3	3.6	4.6	3.1	3.9	4.5
heptanal	8.2	4.4	7.4	11.7	4.6	11.8	6.7	7.7	6.8	6	5.9	7.5	5.7	6.1	8.6	7.9
2,5-dimethylpyrazine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-pentylfuran	3.8	2.7	3.6	6.2	2.3	12.9	4.7	6.7	6.9	2.7	3.1	4.8	4.5	1.9	4.3	3.4

Table A 2: Flavor compounds and their concentrations as detected by headspace sampling in samples containing milk protein and glucose

Protein Source / Sugar	**Dryed non-fat Milk Powder/Glucose**															
Temperature [°C]	140	140	140	140	140	180	180	180	180	180	160	160	160	160	160	160
Protein [%]	1.3	1.3	2	2.6	2.6	1.3	1.3	2	2.6	2.6	2	2	2	1.3	2	2.6
Sugar [%]	2	6	4	2	6	2	6	4	2	6	2	4	6	4	4	4
1-butanol	15.9	13	9.3	3.8	10.1	9.5	5.6	11.3	16.1	12	5.5	5.4	3.1	9.4	4.7	7.8
1-hydroxy-2-propanone	28.4	95.2	98.5	71.1	255	135	149	159	142	214	70.9	121	193	130	170	165
2,3-pentanedione	18.6	7.7	10.5	7	13.1	12.7	6.8	15.1	11.4	11.6	7.5	6.9	10.2	8.2	10.8	8.8
3-hydroxy-2-butanone	24.5	21.8	16.5	8.2	17.9	7.2	9,3	9.3	10.5	10.5	6.4	7.8	11.3	10.2	12.8	8.3
pyrazine	0	0	0	0	0	5.4	4.4	7	9.2	8.5	3.2	2.7	5.5	1.3	6.7	3.4
pentanol	5.7	2.5	3.4	1.8	5.9	1.4	2.6	2.5	2.1	3.2	0.5	2.9	0.9	0.9	2.1	5.1
butyric acid	2.5	8	6.2	3	14.8	7.9	7.5	10.3	10.3	16.2	2.1	4	11.9	2	6.6	6.5
hexanal	93.8	49.1	58.1	36.2	79.1	65.7	49.6	62.7	59.5	62.3	37.2	44.1	52.7	50.3	63.5	47.9
dihydro-2-methyl-3(2H)-furanone	1.5	0	2.8	4.6	6.8	6.2	3.9	12.1	19.6	13.8	4.9	4.3	6.3	4	8.5	6.3
methylpyrazine	0	0	0	0	0	4.2	4.5	6.6	8.8	8.2	0	2.3	5.2	0	5.1	4.2
4-hydroxy-4-methyl-2-pentanone	31.6	11.2	13.1	3.3	10	5.1	5.4	6.9	7.8	8.9	3.6	4.4	6.1	7.5	5.7	12.8
furancarboxaldehyde	15.4	20.7	15.7	10.8	18.9	11.1	9.4	16.7	21.3	22	7.6	9.1	13	11.2	17.8	15.5
furanmethanol	24	8.4	12.5	9.9	13	18.3	8.4	22.4	26.6	32.7	11.2	9.8	16	17.3	22.3	18.8
4-heptanone	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218
3-methyl-2-butanone	9.6	5.4	6.1	3.9	9.9	4.4	5.1	5.7	5.6	8.1	3.1	4.6	7.7	6	6.7	6.3
heptanal	17.9	8.5	9.5	5	9.6	6.9	6	7.1	5.9	9	3.7	6	7	7.4	7.1	6.3
2,5-dimethylpyrazine	7.5	4.8	3.6	1.7	2.2	0	0	0	0	0	0	0	0	0	0	0
2-pentylfuran	11.2	0	5	3	5.6	5.2	2.9	5.4	4.8	4.8	2.7	3.8	4.4	7.4	6.3	5.2

Table A 3: Flavor compounds and their concentrations as detected by headspace sampling in samples containing whey protein and glucose.

Protein Source / Sugar	**Whey Protein/Glucose**																							
Temperature [°C]	140	140	140	140	140	140	180	180	180	180	180	180	160	160	160	160	160	160	160	160	160	160	160	160
Protein [%]	1.3	1.3	2	2.6	2.6	2.6	1.3	1.3	2	2.6	2.6	2.6	2	2	2	2	1.3	2	2.6	2.6	2	2	1.3	2.6
Sugar [%]	2	6	4	2	6	2	6	4	2	6	4	2	6	4	2	6	4	2	6	4	2	6	4	4
1-butanol	3.4	2.8	3.5	5.5	4	4.1	2	2.8	2.5	4.6	0.8	6.2	0.7	2.6	4.1	4.3								
1-hydroxy-2-propanone	36.4	61.2	77	113	117	128	120	12.8	115	166	113	156	119	9.4	138	162								
2,3-pentanedione	9.9	5.1	11	28.6	14.5	12.9	9.2	13.2	18.3	11.7	22.3	22.8	8.2	10.2	14.2	17.9								
3-hydroxy-2-butanone	32.7	19.3	16.4	10.6	8.5	6.3	6.9	5.9	5.6	8.8	5.1	8.2	6.7	6.8	6.6	7.2								
pyrazine	0	0	1	2.3	1.4	4.3	3.2	4.7	6.6	4.5	4.9	5.9	3.5	2.3	5.3	4.5								
pentanol	2.9	2.2	2.9	4.1	2.9	3.8	2.3	2.9	2.8	2.8	3.8	3.6	2.3	1.8	3.4	2.8								
butyric acid	2.5	2.1	3.3	4	4.9	6.4	7.3	7.9	6.6	11.4	3.5	4.5	4.6	1.8	4.9	6								
hexanal	59.2	45.3	61.5	74.3	52.6	61.1	37.5	4.3	45.8	32.1	76.1	67.8	30.7	35.6	55	46.1								
dihydro-2-methyl-3(2H)-furanone	2.5	2	2.6	3.3	2.8	3.7	3	3.1	2.9	3.7	3.8	4.4	2.7	2.8	4.6	4.5								
methylpyrazine	0	0	1.5	2.5	2.2	7.4	5.8	7.5	7.8	9.6	6.5	5.9	5.3	2.2	8	6.5								
4-hydroxy-4-methyl-2-pentanone	7.5	6.4	7	7.1	6.5	9	5.4	5.3	3.5	5	8.6	9.9	3.5	7.2	6.3	8.3								
furancarboxaldehyde	3.3	4.6	4.7	5.4	6.7	5.1	7	6	4.8	9	4.9	8.1	6.4	5.4	7.2	8.2								
furanmethanol	8.5	2.7	4.9	7	6.7	4.5	1.9	3.4	4	2.6	8.5	12.4	3.2	5.9	5.9	6								
4-heptanone	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218	218								
3-methyl-2-butanone	5.1	5.9	6.1	9.3	6	6.1	6.6	6.3	6.6	7.8	8.1	7.8	6	4.7	7.1	8.5								
heptanal	7.5	6.7	8.2	11.3	7.2	7.9	4.2	5.6	7.6	4.4	11.2	10.1	4.3	5.7	7.6	7.5								
2,5-dimethylpyrazine	2.2	0	1.9	4	0	1.9	1.1	2.5	4	2.6	3.5	1.4	0.7	0.8	1.4	1.1								
2-pentylfuran	7.3	2.9	6.1	8.5	6.4	9.2	4.2	5.7	6.6	4	9	10.3	4.4	4.9	8.1	7.8								

Table A 4: Color measurement data obtained from samples containing different combinations and amounts of whey protein or milk protein and glucose syrup or glucose

Sample			b*			a*			L		
Sugar [%]	Protein [%]	Temp. [°C]	MP/CS	MP/G	WP/G	MP/CS	MP/G	WP/G	MP/CS	MP/G	WP/G
2	1.3	140	26.29	23.75	20.83	2.46	3.34	2.35	23.77	27.09	25.01
6	1.3	140	25.92	24.15	21.21	1.85	5.17	4.00	22.27	31.43	29.73
2	2.6	140	28.28	24.77	20.44	3.75	4.68	3.08	26.10	30.26	26.78
6	2.6	140	29.25	24.77	22.51	5.75	6.57	5.09	30.49	34.92	32.79
0	0	160	14.34	17.77	14.34	-3.52	-2.51	-3.52	9.13	12.22	9.13
4	0	160	19.60	20.45	20.89	-2.05	-0.12	0.18	14.63	19.55	20.96
4	0.6	160	23.37	22.47	21.20	1.23	3.08	3.00	21.84	27.58	27.83
4	1.3	160	25.96	22.90	21.13	4.18	4.05	4.30	28.49	29.90	31.83
4	2	160	26.20	23.36	21.66	5.01	4.75	4.55	30.35	31.46	31.60
4	2.6	160	27.76	24.17	21.54	5.00	5.62	5.17	29.12	33.34	33.91
0	2	160	26.05	22.71	16.47	2.91	1.19	-2.30	24.72	20.71	11.61
2	2	160	26.15	22.81	20.72	3.73	2.96	2.48	27.00	26.32	25.90
4	2	160	26.73	24.40	21.84	4.47	5.46	4.46	28.79	32.56	31.77
6	2	160	28.09	24.79	21.62	5.47	5.89	5.89	30.99	33.30	35.55
8	2	160	26.02	24.82	22.39	3.45	7.06	6.84	26.25	36.08	37.41
2	1.3	180	22.85	21.69	20.56	1.83	2.20	2.56	23.36	24.67	26.63
6	1.3	180	25.46	22.95	21.06	3.22	3.93	5.23	25.60	28.90	34.29
2	2.6	180	25.68	22.09	19.50	4.29	2.90	2.71	28.49	27.00	27.42
6	2.6	180	27.37	23.24	20.75	5.94	4.53	5.61	32.23	31.13	35.96

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