INVESTIGATION OF SUGAR REDUCTION IN BISCUITS BASED ON SENSORY ANALYSIS

BİSKÜVİLERDE ŞEKER AZALTMANIN DUYUSAL ANALİZ TEMELLİ İNCELENMESİ

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To my family…

ABSTRACT

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Biscuits are the bakery products frequently preferred by consumers of all ages, as they are easily accessible, ready for consumption, and have a wide variety. Sugar has important effects on taste, texture, and structure. It is a key ingredient in biscuit production and directly related to consumer acceptance. The most used sugar in bakery products is sucrose due to its technological properties and economic reasons. On the other hand, with the proof of its negative effects on health and the increase in the trend of healthy living, sugar reduction strategies in biscuits have gained momentum.

Sugar reduction is a challenge for baking industry. Sensory analysis performed for sugar reduction purposes requires a lot of effort for the industry. Therefore, a modified Weibull model-based approach is presented here to relate sweetness perception with sugar concentration for the first time. The model was tested by using sweetness perception data obtained from sensory analysis of biscuits (with wholewheat flour, whey or hydrolysed pea protein, different forms of sucrose, ethylvanillin, furaneol, and phenylacetaldehyde) having varying sucrose concentrations (6-39%).

Sweetness perception increased with the addition of wholewheat flour, proteins, and aroma compounds. Wholewheat flour and protein addition boosted Maillard reaction products imparting baked/caramel-like flavour notes. No relationship was found between the physical properties of the biscuits and their perceived sweetness.

The modified Weibull model was well fitted to the sweetness perception data with a sigmoidal curve. High predicting power for the model was observed for all biscuits. The model parameters allowed to explain how much sugar reduction can be achieved to reach a targeted sweetness perception without performing further sensory analysis.

Keywords: Sweetness perception, Weibull model, biscuits, sugar reduction, Maillard reaction, aroma

ÖZET

BİSKÜVİLERDE ŞEKER AZALTMANIN DUYUSAL ANALİZ TEMELLİ İNCELENMESİ

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Bisküvi kolay ulaşılabilir olması, tüketime hazır olması ve çok çeşidi bulunması nedeniyle her yaştan tüketicinin sıklıkla tercih ettiği bir unlu mamuldür. Şekerin tat, doku ve yapı üzerinde önemli etkileri vardır. Şeker, bisküvi üretiminde önemli bir bileşendir ve doğrudan tüketici kabulü ile ilgilidir. Unlu mamullerde en çok kullanılan şeker teknolojik özellikleri ve ekonomik sebeplerden dolayı sükrozdur. Öte yandan sağlık üzerindeki olumsuz etkilerinin kanıtlanması ve sağlıklı yaşam trendinin artmasıyla birlikte bisküvilerde şeker azaltma çalışmaları hız kazanmıştır.

Şekerin azaltılması, fırıncılık endüstrisi için bir zorluktur. Şeker azaltma amaçlı yapılan duyusal analizler sektör için çok fazla emek gerektirmektedir. Bu nedenle, burada ilk kez tatlılık algısını şeker konsantrasyonuyla ilişkilendirmek için modifiye Weibull modeline dayalı bir yaklaşım sunulmuştur. Model, değişen sakaroz konsantrasyonlarına (%6-39) sahip bisküvilerin (tam buğday unu, peynir altı suyu veya hidrolize bezelye proteini, sükrozun farklı formları, etilvanilin, furaneol, fenilasetaldehit içeren) duyusal analizinden elde edilen tatlılık algısı verileri kullanılarak test edilmiştir.

Tam buğday unu, proteinler ve aroma bileşenlerinin eklenmesiyle tatlılık algısı artmıştır. Tam buğday unu ve protein ilavesi, fırınlanmış/karamel benzeri lezzet notaları veren Maillard reaksiyon ürünlerini artırmıştır. Bisküvilerin fiziksel özellikleri ile algılanan tatlılıkları arasında herhangi bir ilişki bulunamamıştır.

Modifiye Weibull modeli, sigmoidal bir eğri ile tatlılık algı verilerine iyi bir şekilde uyarlanmıştır. Tüm bisküviler için modelin tahmin gücünün yüksek olduğu gözlemlenmiştir. Model parametreleri, daha fazla duyusal analiz gerçekleştirmeden hedeflenen bir tatlılık algısına ulaşmak için şekerin ne kadar şeker azaltılabileceğini açıklamamıza izin vermiştir.

Anahtar Kelimeler: Tatlılık algısı, Weibull modeli, bisküvi, şeker azaltma, Maillard reaksiyonu, aroma

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SYMBOLS AND ABBREVIATIONS

Symbols

α	the scale parameter of the Weibull model
β	the shape parameter of the Weibull model
C	concentration of sucrose (%)
I	the intensity of the physical stimulus of the Power Law model
k	the scale parameter of the Power Law model
n	the Power Law index
S	the perceived intensity of sweetness
S_i	the initial perceived sweetness
S_e	the equilibrium perceived sweetness
S_m	the upper asymptote of perceived sweetness

Abbreviations

A1	Hexanal
A2	Nonanal
A3	(*E*)-2-Octenal
A4	(*E*)-2-Nonenal
A5	(*E,Z*)-Decadienal
A6	(*E,E*)-2,4-Decadienal
AC	Butanoic acid
AL1	2,3-Butanediol
AL2	(*E*)-2-Octen-1-ol

AL3	Benzenemethanol
AL4	2-Phenylethanol
C	Control biscuit
CY	Cyclotene
F1	First principal component
F2	Second principal component
FR	Furaneol (2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone)
K1	2,3-Butanedione
K2	2,3-Pentanedione
K3	2-Heptanone
K4	2-Octanone
K5	Acetol
K6	3-Octen-2-one
K7	3,5-Octadien-2-one
L1	γ-Valerolactone
L2	γ-Butyrolactone
L3	γ-Crotonolactone
L4	2-Nonenoic acid γ-lactone
LRI	Linear retention index
P1	Pyrazine
P2	2-Methylpyrazine
P3	2,5-Dimethylpyrazine
P4	2,6-Dimethylpyrazine
P5	Ethylpyrazine
P6	2,3-Dimethylpyrazine
P7	2-Ethyl-6-methylpyrazine

P8	2-Ethyl-5-methylpyrazine
P9	2-Ethyl-3-methylpyrazine
P10	Trimethylpyrazine
P11	2,6-Diethylpyrazine
P12	Ethenylpyrazine
P13	2-Ethyl-3,6-dimethylpyrazine
P14	2,3-Diethylpyrazine
P15	2,5-Diethylpyrazine
P16	2-Ethyl-3,5-dimethylpyrazine
P17	2-Ethenyl-6-methylpyrazine
P18	2,3-Diethyl-5-methylpyrazine
P19	2-Ethenyl-5(3)-methylpyrazine
P20	3,5(6)-Dimethyl-2-*n*-propylpyrazine
P21	2-Acetyl-3-methylpyrazine
P22	2-Acetylpyrazine
P23	2-Acetyl-6-methylpyrazine
P24	2-Acetyl-5-methylpyrazine
P25	2-(2'-Furyl)-pyrazine
PC1	Guaiacol
PC2	Phenol
PC3	4-Vinyl-2-methoxyphenol
PC4	4-Vinylphenol
PC5	Vanillin
PCA	Principal Component Analysis
PN1	Maltol
PN2	2,3-Dihidro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one

PP	Pea protein-added biscuit
PR1	1*H*-Pyrrole
PR2	2-Ethyl-4-methyl-1*H*-pyrrole
PR3	2-Acetylpyrrole
PR4	1*H*-Pyrrole-2-carboxaldehyde
PR5	2-Formyl-5-methylpyrrole
PY	2-Acetylpyridine
S1	Dimethyl disulfide
S2	Dimethyl trisulfide
S3	2-Acetylthiazole
S4	2-Thiophenecarboxaldehyde
SA1	2-Methylpropanal
SA2	2(3)-Methylbutanal
SA3	Methional
SA4	Phenylacetaldehyde
SPME	Solid-phase microextraction
WW	Wholewheat biscuit
WP	Whey protein-added biscuit

1. INTRODUCTION

The most widely used sugar in bakery products is sucrose because it is cheap, accessible, and suitable for the final product characteristics. Sucrose has a role both in dough mixing and baking. During dough mixing, sucrose crystals cause the abrasion of oil crystals, provide bubble stability, viscosity, and cohesion to the dough, and prevent gluten network development. Additionally, sucrose controls the water activity, evaporation rate, and vapor pressure, promotes spreading, delays gluten denaturation, and prevents starch gelatinization at high temperatures during baking [1]. The decreases in the amount of sucrose cause significant changes in the shelf life of bakery products and the quality characteristics such as colour, volume, and texture [2,3]. Despite the significant role of sucrose in bakery products, excessive sucrose consumption has some negative effects on health such as the increased risk of obesity, type-2 diabetes, and cardiovascular disorders [4]. Therefore, both the industry and academia have focused on sugar reduction in bakery products in recent years.

Adding non-nutritive sweeteners, sugar alcohols, and fibres are common strategies for sugar reduction in bakery products [5]. In addition, changing the particle size of the added sugar [6], the distribution of sugar in different layers of food [7], and using flavouring substances as sweetness enhancers [8] are also considerable approaches. For instance, sugar reduction was shown to be possible in muffins with the addition of vanillin [7]. Maltol was found to increase the sweetness of sucrose solution in a panel performed by untrained panellists [8].

It is important to determine the change in the perception of sweetness in sugar reduction studies. By performing a set of sensory analyses for certain sugar concentrations, the change in perception versus the change in sugar concentration can be graphically represented and explained with a mathematical model. This would answer the question of how changes in sugar concentration for the same product would affect the perception of sweetness without the need for further sensory analysis for all sugar concentrations.

Stevens power law, which is expressed by following equation

$$S = kI^n$$

where S is the perceived intensity of a sensory attribute, I is the intensity of the physical stimulus, k is the scale parameter, and n is the power law index, is frequently used to explain the relationship between sugar concentration and sweetness perception [9–12]. However, its application becomes questionable in the case of two or more stimuli involves in the perception of the sensory attribute [13] which is the case in most food matrices. More importantly, it is not capable of predicting the nonlinear subregion of sweetness perception when a sigmoidal curve is obtained for sweetness perception versus sugar concentration.

The Weibull model is a probabilistic model which is used to explain various concepts in different food matrices, such as soaking of breakfast cereals in milk [14], water uptake of dehydrated carrots [15], and shelf-life estimation of foods [16,17]. However, the Weibull model, which has been effective in elucidating many concepts in food science, has not been used in sugar reduction studies before, as far as we know. An S-shape curve provided by the Weibull model may help defining the lower and upper limits at which the panellists can no longer perceive changes. Applying the Weibull model to the sweetness perception data of bakery products can make it easier to mathematically express panellists' responses to changes in bakery recipes. Therefore, the aim of this thesis was presenting a new tool for understanding how the perception of sweetness changes at varying sucrose concentrations in biscuits with different recipes using a modified version of the Weibull model.

2. GENERAL INFORMATION

2.1. The role of sugar in biscuits

Sugar, one of the main components of biscuits, has critical effects on biscuit structure. In addition to giving taste, it affects many quality features of the biscuit and interacts with other basic biscuit components [17].

The most preferred sugar in bakery products is sucrose because it is cheap, accessible, and suitable for the final product characteristics. Sucrose affects the taste, texture, dough stability, colour, volume, shelf life, and fermentation properties of baking goods [2]. In addition to being responsible for the sweet taste, sugar is also used to mask the off flavour [17].

During dough mixing, sucrose crystals cause the abrasion of oil crystals, provide bubble stability, viscosity, and cohesion to the dough, and prevent gluten network development. Additionally, sucrose controls the water activity, evaporation rate, vapour pressure, and promote spreading, delay gluten denaturation, and prevent starch gelatinization at high temperatures during baking. Moreover, degradation of the reducing sugar during baking contributes the formation of the desired browning and flavour compounds [18]. In addition, sucrose partially dissolved during baking solidifies when the biscuit cools, creating a hard and glassy structure [19]. Therefore, decreases in the sucrose content of baking goods cause significant changes in their taste, texture, colour, volume, and shelf life [20].

The interaction of sugar with other biscuit components has important consequences in terms of consumer acceptance. There is no chemical reaction between sucrose and fat, but the rheological properties of the product may change as a result of the physical interaction of these two important components. In addition, the gelatinization temperature of the starch in the formulation increases as the sugar concentration increases. The reason for this situation is that the affinity of sugar for water is higher than that of starch, so as the sugar concentration increases, the water required for the gelatinization of starch decreases in the medium. The height, spreading rate, and surface properties of the biscuit are affected by this increase. Moreover, Strecker degradation and Maillard reaction occur as a result of the interaction of reducing sugars with amino compounds. As a result of these reactions, which proceed with different mechanisms and produce different end products, the desired flavour, aroma, and colour of biscuits are formed [17].

2.2. Strategies to reduce sugar content in biscuits

The negative effects of excessive sucrose consumption on health, such as the risk of obesity, type-2 diabetes, and cardiovascular disorders led industry and academia to focus on sugar reduction in bakery products [4]. It is a great challenge to reduce the amount of added sugar in these products due to their unique properties that provide to the dough and the final product [21].

Research on sugar reduction in bakery products is highly popular in recent years. Sugar reduction studies focus on two main approaches: using sugar substitutes and gradual sugar reduction.

Adding non-nutritive sweeteners, sugar alcohols, and fibres is a common strategy in most of the sugar reduction studies focused on using sugar substitutes [5]. In addition, changing the particle size of the added sugar [6], the distribution of sugar [22,23], and using flavouring substances as sweetness enhancers [24] are also considerable approaches for sugar reduction.

di Monaco et al. [25] suggested that maltitol, erythritol, inulin, and stevioside-maltodextrin can be used as sugar substitutes for sucrose and sucrose-citric acid solutions. Tyuftin et al. [6] found that the particle size of added sugar affects the sweetness perception and the physical properties of the final product. According to their study, biscuits prepared with coarse ground sugar (228-377 μm) have higher scores of sweetness perception and consumer acceptance than biscuits prepared with finely ground sugar (124 to 179 µm). Mosca et al. [23] determined that the perceived sweetness score increased when gels with the same sucrose concentration and similar textural and rheological properties showed inhomogeneous sucrose distribution. With this inhomogeneous sucrose distribution method, small changes in sucrose concentration are not noticed by the consumers and it is possible to reduce by 20% sucrose [23]. In addition, Mosca et al. [22] investigated the effect of the mechanical properties of the gel layers, in which sucrose is inhomogeneously dispersed, on the perceived sweetness. They found that enhancement in sweet taste by inhomogeneous sucrose distribution does not depend on the soft or hard texture of the gel matrix. However, they also determined that because gel texture affects oral processing, soft gels are perceived as the sweetest and hard gels are perceived as the least sweet [22]. Moreover, sugar reduction is possible in muffins by the addition of 1% vanilla [26] and with maltol-sucrose interaction [24]. Although various strategies have been tested to reduce the amount of added sugar in biscuits so far, a desirable strategy that provides consumer acceptance or technological properties could not be obtained yet [27].

To sum up, considering all these studies, it can be thought that sucrose substitutes, adding flavouring agents, and changes in the physical properties of added sucrose are promising strategies to reduce sugar in baking goods. However, it is known that reducing sucrose in sweet bakery products reduces sweetness [28] and tenderness, leads to a gummy and chewy texture [29], and causes a less porous [30] and less viscous [31] structure. Moreover, it was observed that in the sugar-reduced biscuits that the crust colour remained lighter [32], fewer cracks were formed on the surface [33], and the biscuits were less crispy [34]. Therefore, reducing sugar in biscuits is still a challenge.

On the other hand, in gradual sugar reduction strategies, small changes are made that will not change the characteristics of the products and consumer perception, and the consumer is expected to get used to this new sweetness intensity for a while. In this way, the work is the work is carried out on over a long period of time and with repeated steps [35].

Lima et al. [36] reported in the gradual sugar reduction in grape nectar study that it is possible to reduce sugar in products appealing to children at 6-12 years old through gradual sugar reduction without loss of acceptance or difference in perceived sweetness. Velázquez et al. [37] additionally, suggested that gradual sugar reduction in vanilla milk desserts can be achieved without changing overall acceptibility acoording to the results of sensory analysis performed by children. Oliveira et al. [38] stated that with gradual sugar reduction in chocolate milk, it is possible to reduce sugar by 6.7% without changing the consumer perception and the characteristics of the product.

However, this method is difficult and laborious to implement, since results are obtained in a long time loop, a standard product, and consumer feedback is needed. Therefore, it was recently stated that more studies are needed on the subject [35].

2.3. Reactions that provide flavour formation in biscuits

2.3.1. Maillard reaction

The Maillard reaction occurs between reducing carbohydrates and amino acids or proteins in food matrices during thermal treatments [39]. The Maillard reaction is very important in the formation of the quality characteristics of heat-treated foods, such as browning, nutritional value, and flavour formation. Various aroma compounds are formed with the Maillard reaction, while the type of sugar and amino acid determines the type of the compounds. On the other hand, reaction kinetics depend on temperature, time, pH, and water content [40].

As a result of the Maillard reaction, pyrazines with baked, roasted, fried, oven-roasted flavour, alkylpyrazines with a nutty, and roasted flavour, alkylpyridines with bitter, burnt, astringent flavour, and acylpyridines with cracker-like flavour are formed. Moreover, pyrroles imparting cereal-like flavour, furans, furanones, and pyronones with sweet, burnt, pungent, caramel-like aroma, and oxazoles with green, nutty, sweet aroma are formed by the Maillard reaction [40]. The formation mechanism of these aroma compounds is shown in **Figure 2.1**.

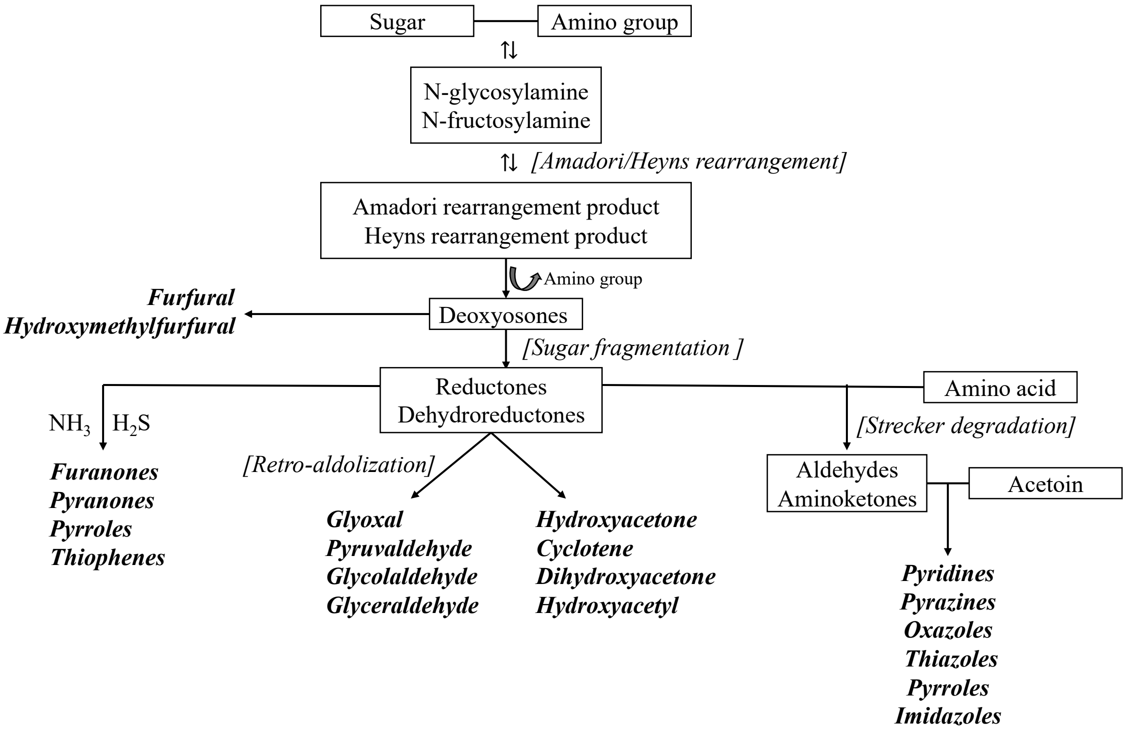


Figure 2.1. Pathways of aroma compounds formation in Maillard Reaction, adopted from [40].

Maillard reaction is very important in biscuits in terms of aroma profile and flavour. 2-Methylbutanal, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, and benzaldehyde, which are the compounds that give the biscuit its characteristic aroma, are formed as a result of this reaction. Furan derivatives, which are associated with a caramel-like and sweet aroma, also occur with the Maillard reaction. 2-Methylfuran, 2-pentylfuran, 2-furanmethanol, furfural, 5-methylfurfural, 2-acethylfuran are important furan derivatives that affect the biscuit flavour. Pyrazines such as 2-methylpyrazine and 2-ethylpyrazine, which are effective in the formation of the characteristic flavour of the biscuit, also occur as a result of this reaction [41].

2.3.2. Strecker Degradation

Strecker degradation is one of the most important reactions in terms of aroma formation. In this reaction, as a result of the interaction of α-dicarbonyl compounds with amino acids, Strecker aldehydes are formed, which have a low odour threshold and are responsible for the formation of the characteristic flavour of foods [42], shown in **Figure 2.2**.

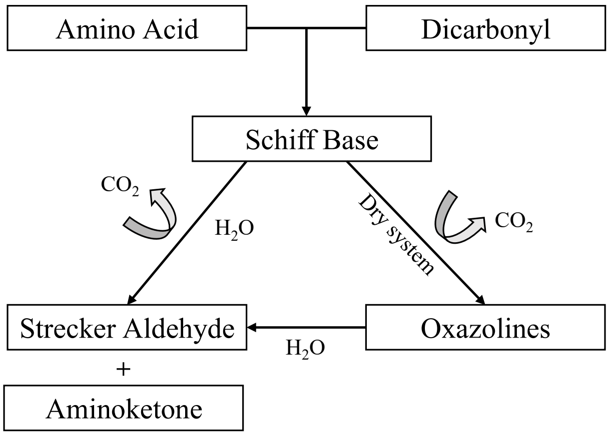


Figure 2.2. Strecker degradation, adopted from [42].

The Strecker degradation of valine gives 2-methylpropanal, leucine gives 3-methylbutanal, and isoleucine gives 2-methylbutanal, the compounds responsible for the malty flavour in biscuits. As a result of Strecker degradation, phenylalanine forms phenylacetaldehyde with honey flavour and methionine forms methional, which is responsible for the cooked flavour [42].

2.3.3. Caramelization

Caramelization is a reaction that occurs when polyhydroxy carbonyl compounds, such as sugars, are heated to high temperatures without amino compounds. Reactions during the Maillard reaction such as furfural formation, sugar fission, and enolization are also observed during caramelization [39]. Depending on the type of polyhydroxy carbonyl, pH, presence of acid, alkali, and salt, various colour and flavour compounds form [43].

In the first step of the caramelization reaction, the dehydration of sugars takes place. Dehydrated sugars condense or polymerize as the reaction progresses. At the initial stage of caramelisation, lightly coloured and pleasant-tasting caramel flavours are produced.

However, as the reaction progresses, the molecular weight and bitterness of the products increase, and their colour darken [43].

During biscuit baking, when the temperature rises above 160ºC, sucrose is hydrolyzed to glucose and fructose with the hydrogen ions it takes from the water in the environment. Afterwards, it passes through stages such as enolization, elimination and dehydration and turns into furan derivatives responsible for the formation of caramel colour and aroma in biscuits. The main compounds with caramelic aroma are furfural, 5-methyl furfural, and 5-hydroxymethyl furfural in biscuits [44].

2.3.4. Lipid Oxidation

Lipid oxidation, which can occur enzymatically or non-enzymatically, causes colour and aroma formation, and changes in taste in biscuits. This reaction can occur through many different mechanisms such as autoxidation, photooxidation, or enzymatic oxidation. Lipid oxidation takes place in three stages: free radical formation, propagation, and termination. As a result of these reactions, hydroperoxides, which are tasteless, odourless, and unstable primary oxidation products, as well as secondary oxidation products such as aldehydes, ketones, and carbonyl compounds, which affect the flavour of the food, are formed [45]. In addition, the interaction of lipid oxidation products with amine compounds is associated with browning products that occur during food processing and storage. Moreover, it is thought that lipid oxidation products promote the Maillard reaction and react with some Maillard Reaction intermediates to form aroma compounds [46]. Negroni et al. [47] investigated the effects of lipid oxidation products on Maillard reaction using olive oil, canola oil, and sunflower oil. They reported that the formation of 2-methylpyrazine, 2,5-dimethylpyrazine, and 2,3-dimethylpyrazine, which are Maillard reaction products, increased with the increase in lipid oxidation.

Lipid oxidation products from wheat flour in biscuits are also very effective on flavour. Compounds such as hexanal, octanal, *(E)*-2-octanal, nonanal, *(E)*-2-nonanal are formed in this way. In addition, important volatile compounds such as benzaldehyde or 2-

pentylfuran can be formed as a result of lipid oxidation as well as the Maillard reaction [41].

2.4. Determination and modelling of sweetness perception in biscuits

Sensory analysis is a method that allows measuring people's reactions to foods by minimizing consumer biases and effects. Common methods used for sensory analysis are difference testing, descriptive analysis, and effective testing. Scaling methods are used to measure the sensory evaluations obtained by these methods and turn them into numerical data that can be used in processes such as statistical analysis, modelling, estimation, and comparison. The most common methods for scaling are line, and category scales or magnitude estimation. Line scaling is a method for scaling intensity and is applied by placing a mark on a line that expresses the examined feature. Results are obtained by measuring the distance of the marked point from one end of the scale. It is widely used because it is easy to apply and sensitive to the differences in product [48].

Different sensory analysis methods can be used to correlate sugar concentration with sweetness intensity in various products. For example, McBride [49] used the category scale to describe the sweetness of sucrose, fructose, and glucose and to calculate the sweetness values corresponding to their concentrations. Abdallah et al. [50] collected the panellists' reactions to the sweetness of commercial cakes and biscuits on a 9-point hedonic scale from "not at all sweet" to "extremely sweet" and interpreted the results statistically with linear and multilinear regression. James et al. [51]conducted a trained magnitude estimation panel in their study of both children and adults participated and they correlated the sweetness intensity with the sucrose concentration in sucrose solution, orange drink, custard, and biscuit. McBride et al. [52] adopted the magnitude estimation method to evaluate the sweetness intensity of lemon drinks containing mixtures of fructose and sucrose in different proportions. Biguzzi et al. [53] studied the sweetness intensity of reduced sugar biscuits, and they presented the panellists a 5-point intensity scale from "not sweet at all" to "extremely sweet". Using the triangular test and pairwise comparison to describe the sensory sweetness of different sugars, Mao et al. [54] created a model to calculate the sweetness indices corresponding to various sugar concentrations of sucrose, glucose, fructose, lactose, maltose, and combinations of these sugars. Torrico et al. [55], on the other hand, used the consumer rejection threshold method to determine the perceived sweetness of strawberry-flavoured yoghurts containing different concentrations of sucrose.

It is possible to find the sweetness perception corresponding to different sucrose concentrations by performing sensory analysis. However, testing the effect of sugar reduction on the sweetness perception of products could be impractical for the changes in sugar concentration in small intervals due to various challenges faced in sensory analysis such as the willingness of the panellists and economic limitations. Therefore, it is necessary to explain the observed sensory analysis data with a mathematical model.

Steven's Power Law is a frequently preferred function in the literature to explain the relationship between sugar concentration and sweetness [56–58]. According to the results of sensory analysis, the effect of the change in sugar concentration on sweetness is estimated by the following equation

$$S = kI^n$$

where S is sensory intensity, I is physical intensity, and k is the scale parameter. k characterizes the conversion of the stimulus ratio to the sensory ratio and is usually fixed to 1.3 for the relationship between sugar concentration and sweetness perception [56]. It has been reported that the k value may vary from experiment to experiment, and it has been calculated as 1.3 [11], 1.0 [12], 0.75, and 0.6 [59] in previous studies of sugar concentration and sweetness relationship. In addition, the sweetness perception curves obtained using this model do not show an s-shape curve contrary to expectations and are insufficient to estimate the upper limit.

The modified Weibull model is one of the widely used empirical models that allow to explain various concepts in food science, such as soaking of cereals in milk [60], and the shelf life of dehydrated carrots [61], etc. However, it was not considered to be applied to the sweetness perception of bakery products before. Application of the modified Weibull model to the sweetness perception of bakery products could easily explain the reactions of panellists to the changes in the recipe of the bakery products mathematically. In the sweetness perception of bakery products, the modified Weibull Model [60] could be given as follows:

$$S = S_i + (S_e - S_i) \times [1 - exp\left(-\left(\frac{C}{\alpha}\right)^{\beta}\right)]$$

where S is the perceived sweetness at the C concentration of sucrose, S_i is the initial perceived sweetness, S_e is the equilibrium perceived sweetness. α is the scale parameter and represents the required sucrose concentration to detect 63% $(1 - e^{-1})$ of the equilibrium sweetness perception. β is the shape parameter, and the higher its value, the longer the lag phase suggested by the model [60].

3. MATERIALS AND METHODS

3.1. Chemicals and consumables

Refined wheat flour, shortening, icing sugar, skimmed milk powder, sodium bicarbonate, ammonium bicarbonate, high-fructose corn syrup, whole wheat flour, ethylvanillin, granulated sugar, sodium chloride, and whey protein were purchased from a local store in Ankara. Hydrolysed pea protein RadiPure (80%) was obtained from Cargill (Turkey). Furaneol (≥98, food grade), phenylacetaldehyde (≥95, food grade), C_5-C_{22} alkane mixture, and 3-methyl-2-butanone (≥98.5) were purchased from Sigma-Aldrich (Missouri, USA). Isopropylpyrazine (≥98) was obtained from Santa Cruz Biotechnology Inc. (Texas, USA).

3.2. Preparation of biscuits

Biscuits were prepared according to the American Association of Cereal Chemists Method 10-54 with slight modifications [62]. The dry mixture consisting of 42 g sucrose, 1 g skimmed milk powder, and 1 g sodium bicarbonate was mixed with 40 g shortening at 20 °C for 1 min in Kitchen Aid 5KSM150 (Michigan, USA). Then, 1.5 g high-fructose corn syrup, 0.6 g sodium chloride, and 0.5 g ammonium bicarbonate were added to the mixture after dissolving them in 22 g of water (aqueous mixture). After then, the mixture was mixed for 1 min. Finally, 100 g of refined wheat flour was added to the mixture, and it was mixed for 30 s. After each step, the mixture splashed to the sides of the bowl was scraped off with a silicone spatula. The dough was rolled out with a rolling pin to a thickness of 5 mm and cut with a 5 cm round mould. A total of 12 biscuits in each set were baked on a mesh baking mat in a Memmert UNE 400 (Germany) oven at 205 °C for 11 min and cooled on a counter for half an hour.

Half of the refined wheat flour was replaced with wholewheat flour to obtain wholewheat biscuits. In low-fat biscuits, 22 g shortening, and 118 g refined wheat flour were used in the recipe. Whey protein-added biscuits and pea protein-added biscuits were prepared by the replacement of 2 g of refined wheat flour with whey protein mix or pea protein hydrolysate, respectively. Ethylvanillin-added biscuit was prepared by the addition of 0.5 g ethylvanillin to the dry mixture. Furaneol-added biscuit and phenylacetaldehyde-added biscuit were prepared by adding furaneol (1.5 µg/mL) or phenylacetaldehyde (1 µg/mL) to the aqueous mixture mentioned above, respectively. In granulated sugar biscuits and inhomogeneous biscuits, granulated sugar was used instead of icing sugar. In inhomogeneous sucrose distribution biscuits, half of the granulated sugar was added to the dry mixture (creaming), and half of it was added to the refined wheat flour (dough formation).

Biscuits with different sucrose concentrations at 6, 8, 12, 17, 22, 28, 34, 37, and 39% in the dry matter were prepared for each type of biscuit, and the samples were pooled after baking in two batches for the sensory analysis. Control, wholewheat, whey protein-added, and pea protein-added biscuits (at 22% sucrose) were baked in triplicate for volatile compound analysis.

3.3. Physical properties of biscuits

The weight of the biscuits was measured 30 min after they came out of the oven, with a balance. The diameter and height of the biscuits were measured with a digital calliper. The diameters and heights were measured three times from different locations of each biscuit and then the average values were recorded.

3.4. Texture analysis

The hardness of biscuit doughs was tested for 2 mm/s test speed and 5 mm sample height with a 1 cm diameter cylindrical probe in LLOYD Instruments TA Plus Ametek Texture Analyser (Bognor Regis, England). Measurements were repeated twice and hardness values were reported in Newton.

3.5. 3-Point bend test

The hardness of biscuits was tested on a 12.5 mm pitch table by using 2 mm/s test speed and 1 mm/min datum speed in LLOYD Instruments TA Plus Ametek Texture Analyser (Bognor Regis, England). Measurements were repeated twice and hardness values were reported in Newton.

3.6. Colour analysis

The digital images of the biscuit samples were obtained with the image acquisition box prepared using two light sources directed at an angle of 45°C to the sample on a white background. L*, a*, b* colour values, and browning ratio values of biscuits were acquired by using Image Analysis in MATLAB as described by Gökmen & Sügüt [63] and Mogol & Gökmen [64] respectively. L* refers to the luminance, represents the colours from black to white, and takes a value in the range of 0-100. a* represents the colours from green to red, and b* represents the colours from blue to yellow, and these values can range from -120 to 120 [63].

3.7. Gas chromatography-mass spectrometry (GC-MS) analysis of volatile compounds

Ground biscuit samples (3 g) were weighed into 20 mL screw-cap headspace vials. To have the same ionic strength and to make the transfer of the volatile compounds to headspace easy, 3 mL of saturated NaCl solution (35%) was added to the sample and mixed for 30 s with a vortex mixer. Solid-phase microextraction (SPME) of volatile compounds was performed by adsorption on CAR/PDMS/DVB adsorbent fibre by using the SPME-Arrow module of Thermo TriPlus RSH autosampler and then the injections were performed on Thermo Trace 1300 gas chromatography coupled to Thermo ISQ single quadrupole mass spectrometer (Thermo Fisher Scientific Corp., Massachusetts, USA). The vials were equilibrated at 60 °C for 10 min and extracted at 60 °C for 30 min with an agitation speed of 300 rpm. The adsorbent fibre was conditioned at the fibre conditioning unit at 230 °C for 3 min before extraction. The injection was splitless and the desorption was at 230 °C for 1 min. The carrier gas was helium at a flow rate of 1.2

mL/min. The column used for the analysis of volatile compounds was TG-WaxMS column (30 m × 0.25 mm × 1.4 µm film thickness, Thermo Fisher Scientific Corp., Massachusetts, USA). The oven temperature was held at 30 °C for 5 min and then increased from 30 to 230 °C at a speed of 5 °C/min and hold at 230 °C for 10 min. The mass spectrometer was operated in electron ionisation mode (70 eV), the source temperature was 250 °C, and the mass scanning range was m/z of 29 to 400.

The peaks were identified by comparing their mass spectra with the Wiley 9 library and by comparing their linear retention indexes (LRI) with the literature. To achieve that, 1 µL of 100 µg/mL C5-C22 alkane mix was put into a vial and analysed under the same chromatographic conditions. Isopropylpyrazine (for pyrazines) at a concentration of 0.05 mg/mL and 3-methyl-2-butanone (for all other detected compounds) at a concentration of 0.5 mg/mL, both in saturated NaCl solution, were used as internal standards. Relative amounts of the volatile compounds were calculated by using the response factor of 1 for isopropylpyrazine or 3-methyl-2-butanone.

3.8. Sensory analysis

Sensory analysis was performed for biscuit samples containing different sucrose concentrations for each biscuit recipe. A sensory panel of 25 participants (aged 22-55, 15 female and 10 male) with previous sensory analysis experience was used. Sensory panels were organized as two sessions with 30 min break in between, in which 5 biscuits were presented in each session. A quarter of a biscuit (2 g approx.) was packed in a zip lock bag and labelled with a 3-digit random code and presented to each panellist in random order. All biscuits were assessed in duplicate in separate days. Due to the Covid-19 pandemic conditions, the biscuits prepared for the panel were packaged with the panel forms to be used and delivered to the panellists with the necessary directions to ensure the panel conditions were applied in their private areas. The panellists were asked not to be fully hungry or full, not to consume anything other than water for half an hour before tasting, to focus only on sweetness by ignoring the appreciation of the samples, not to compare the samples with each other, to taste the biscuits according to the order in their panel forms, to take the packed quarter of a biscuit in their mouths once and chew, to mark the perceived sweetness of the biscuit on a line scale, to rinse their mouths with plenty of water at room temperature, and to wait for 30 s before moving on the next biscuit. A 10 cm printed line scale anchored with ‘not sweet at all’ and ‘highest imaginable sweetness’ at the ends of the line was used for scoring.

3.9. Modelling of sensory analysis data and statistical analysis

The mean values of perceived sweetness for biscuits with different sucrose concentrations were confronted with the modified Weibull Model in MATLAB by using nonlinear curve fitting. Differences among the volatile compounds of biscuits were determined by using the one-way ANOVA and Tukey posthoc test, and the differences between sweetness scores of the biscuits were compared with randomised block design ANOVA, by using SPSS both at 95% confidence interval. The principal component analysis (Pearson correlation) was carried out using XLSTAT software Version 2022.4.1 (Addinsoft, Paris, France).

4. RESULTS AND DISCUSSION

4.1. Changes in sweetness perception by recipe modifications

4.1.1. Effect of compositional changes

To express sweetness perception with a mathematical model, a series of sensory analyses was performed with different types of biscuits (wholewheat biscuits, low-fat biscuits, whey protein-added biscuits, and pea protein-added biscuits) containing sugar in various concentrations (6-39%), and the sweetness perception data were presented in **Table 4.1**. No difference was found (*$p>0.05$*) in the perceived sweetness of wholewheat biscuits, low-fat biscuits, whey protein-added biscuits, and pea protein-added biscuits compared to control biscuits at sugar concentrations of 6, 8, and 12%. Wholewheat biscuits had higher sweetness scores than control and low-fat biscuits at sugar concentrations between 17-39%. The reason for the higher sweetness scores of wholewheat biscuits might be the higher free amino acid content of whole wheat flour compared to refined flour [65]. Free amino acids are critical for the formation of flavour compounds via the Maillard reaction during baking [66]. The changes in the aroma compounds by recipe modifications are given in section 4.3. Additionally, wholewheat flour increases the content of fibre in biscuits which may change the perception of sweet taste. However, there is no consensus in the literature on the effect of fibre on sweetness perception. Canalis et al. [67] did not find any effect of adding fibre other than inulin on the sweetness perception of the biscuits.

Although an increasing trend of sweetness was observed in whey or pea protein-added biscuits compared to the control, there were only a few statistically significant results. Whey protein-added biscuits containing 17% sucrose, and whey and pea protein-added biscuits containing 39% sucrose had significantly higher sweetness scores. The effect of protein addition on the sweetness perception of biscuits could be due to the increase in volatile compounds formed as a result of the Maillard reaction given in section 4.3.

Lowering the fat content of biscuits did not affect the sweetness perception compared to the control ($p>0.05$). Contrary to our findings, Drewnowski et al. [28] suggested that the perception of sweetness can be masked by increasing the fat content of biscuits.

4.1.2. Effect of physical characteristics of sucrose

Perceived sweetness increased slightly in granulated sugar biscuits at 6% and 8% concentrations compared to inhomogeneous sucrose distribution and control biscuits ($p<0.05$). However, there was no difference in sweetness perception at other sugar concentrations ($p>0.05$) (**Table 4.2**). Similarly, Molina et al. [68] found no difference in the perceived sweetness of biscuits depending on the granule size of sugar. On the contrary, Tyuftin et al. [6] reported an increase in the perceived sweetness of shortbread biscuits when coarsely ground sugar was used. Sugar reduction by 20% without changing the sweetness intensity was reported when an inhomogeneous sucrose distribution was used in a gel matrix [23]. Moreover, Caporizzi et al. [7] reported that muffins produced with inhomogeneous spatial sucrose distribution were perceived as sweeter than those with homogeneous sugar distribution. In this study, the reason why sweetness could not be increased with inhomogeneous sucrose distribution was that biscuits with inhomogeneous sucrose distribution did not consist of layers with different sucrose concentrations.

Table 4.1. Sweetness perception scores of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4±0.4^{ab}	0.7±0.7^{ab}	1.4±1.1^{ab}	2.7±1.9^{b}	4.8±2.0^{bc}	6.8±2.2^{b}	7.5±1.8^{b}	7.6±2.2^{b}	7.9±2.1^{c}
Wholewheat biscuit	0.5±0.6^{ab}	0.9±0.9^{a}	1.9±1.6^{a}	4.2±2.0^{a}	6.5±1.6^{a}	7.9±1.6^{a}	8.7±1.2^{a}	8.8±1.1^{a}	8.7±1.6^{ab}
Low fat biscuit	0.2±0.4^{b}	0.4±0.5^{b}	0.9±1.3^{b}	2.8±2.2^{b}	4.3±2.3^{c}	6.3±2.1^{b}	7.2±2.2^{b}	7.8±2.2^{b}	8.1±2.1^{bc}
Whey protein-added biscuit	0.7±1.1^{a}	0.8±0.8^{a}	1.8±1.6^{a}	4.3±2.2^{a}	5.4±1.6^{b}	6.9±1.9^{b}	7.8±1.8^{b}	8.3±1.5^{ab}	8.7±1.4^{ab}
Pea protein-added biscuit	0.6±0.8^{ab}	0.9±0.9^{a}	1.8±1.3^{a}	3.4±1.9^{ab}	5.4±1.9^{b}	6.4±1.9^{b}	7.8±1.5^{b}	8.2±1.8^{ab}	8.8±1.1^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.2. Sweetness perception scores of biscuits with different physical forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4±0.4^{b}	0.7±0.7^{b}	1.4±1.1^{a}	2.7±1.8^{a}	4.8±2.0^{a}	6.8±2.2^{a}	7.5±1.8^{a}	7.6±2.2^{b}	7.9±2.1^{a}
Inhomogeneous sucrose distribution biscuit	0.4±0.3^{b}	0.7±0.6^{b}	1.8±1.6^{a}	3.3±1.8^{a}	5.0±2.3^{a}	6.5±1.9^{a}	8.0±1.6^{a}	8.4±1.7^{a}	8.5±1.6^{a}
Granulated sugar biscuit	0.7±1.0^{a}	1.3±1.3^{a}	1.6±1.4^{a}	3.0±1.9^{a}	5.1±1.9^{a}	6.6±2.0^{a}	7.5±2.3^{a}	8.1±2.1^{ab}	8.2±1.8^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.3. Sweetness perception scores of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4±0.4^{b}	0.7±0.8^{a}	1.4±1.1^{a}	2.7±1.9^{b}	4.8±2.0^{b}	6.8±2.2^{a}	7.5±1.8^{b}	7.6±2.2^{b}	7.9±2.1^{b}
Ethylvanillin-added biscuit	0.8±0.9^{a}	1.0±0.9^{a}	2.1±1.7^{a}	3.7±1.9^{a}	5.8±1.9^{a}	7.1±1.9^{a}	8.2±1.5^{a}	8.0±1.8^{ab}	8.6±1.3^{ab}
Furaneol-added biscuit	0.4±0.4^{b}	1.1±1.1^{a}	2.1±1.8^{a}	3.8±1.9^{a}	5.6±2.0^{a}	7.5±1.8^{a}	8.2±1.7^{a}	8.7±1.4^{a}	8.8±1.1^{a}
Phenylacetaldehyde-added biscuit	0.5±0.9^{b}	0.9±1.2^{a}	1.7±1.5^{a}	3.5±2.0^{ab}	5.6±1.8^{a}	7.1±1.4^{a}	8.1±1.6^{ab}	8.6±1.2^{a}	8.7±1.2^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at $p=0.05$.

4.1.3. Effect of addition of flavour compounds

Ethylvanillin has sweet, creamy, vanilla-like, and caramel flavour. Furaneol has sweet, caramelized, burnt sugary, maple, cotton candy, and fruity flavours. Phenylacetaldehyde imparts a honey-like, floral, and sweet flavour. Therefore, the addition of ethylvanillin, furaneol, and phenylacetaldehyde might affect the perceived sweetness at various concentrations. Ethylvanillin and furaneol-added biscuits were perceived sweeter than control biscuits at 17%, 23%, and 34% sucrose concentrations, and phenylacetaldehyde-added biscuits were perceived sweeter than control at high sucrose concentrations (34-37%). However, no significant difference ($p>0.05$) was found in the perceived sweetness of the flavour-added biscuits at lower sucrose concentrations (6-12%) compared to the control except for ethylvanillin-added biscuit at 6% sucrose concentration where a higher sweetness was perceived (**Table 4.3**).

Hence, adding flavourings associated with sweetness to the biscuit formulation caused an increase in perceived sweetness intensity depending on the sucrose concentration. Similarly, Bertelsen et al. [69] studied the effects of various flavourings on sweetness intensity at different sucrose concentrations and reported that vanilla, honey, and banana flavours increased the sweetness perception at low and medium sucrose concentrations, on the other hand, they did not find a significant difference at high sucrose concentrations. Caporizzi et al. [7] reported that adding 1% vanillin had a synergistic effect with fine sugar particles in muffins and increased the sweetness intensity, which was also consistent with our data. However, it was reported that this effect depends on the food matrix and the amount of flavouring used [7]. Additionally, Lavin et al. [70] reported that adding vanilla extract increased the perceived sweetness in milk. Kulka [71] found that ethyl vanillin was very effective as an aroma booster, and Noble [72] reported that vanillin acted as a sweetness enhancer by showing a synergistic effect with sucrose.

4.2. Modelling sweetness perception by a modified Weibull model

A modified version of the Weibull model was applied to the sweetness perception scores of biscuits. Weibull Model can be modified to explain sweetness perception as follows:

$$S = S_i + (S_m - S_i) \times [1 - exp\left(-\left(\frac{C}{\alpha}\right)^{\beta}\right)]$$

where S is the perceived sweetness at the C concentration of sucrose, S_i is the lower asymptote of perceived sweetness which at minimum can be 0 (not sweet at all), S_m is the upper asymptote of perceived sweetness, which at maximum can converge to the highest value of a sensory scale. α is the scale parameter and represents the required sucrose concentration to obtain 63% $(= 1 - e^{-1})$ of $S_m - S_i$. β is the shape parameter.

Average values of sweetness perception scores (also called observed values that are shown with markers in **Figures 4.1-3**) were plotted against sucrose concentrations. The modified Weibull model, predicting the data, was presented with lines in **Figures 4.1-3**. The model was also tested by modifying the biscuit recipe (type of flour, different proteins, and amount of fat), changing the physical form of sucrose, or adding aroma compounds. The sweetness perception showed a sigmoidal curve with changing sucrose concentration and the Weibull model was visually well-fitted to the data indicating the suitability of the model for the prediction of sweetness perception in all biscuit recipes. Additionally, the relative 95% confidence intervals of model parameters given in **Table 4.4** were mostly below 20%, indicating an acceptable predicting power of the modified Weibull model for the perceived sweetness in biscuits, except for S_i.

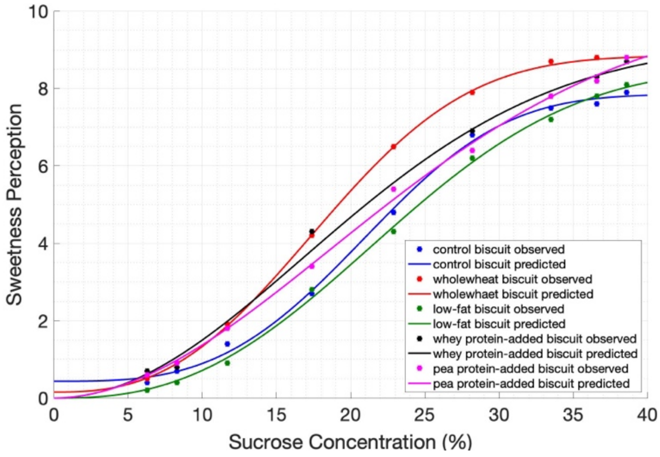


Figure 4.1. Sweetness perception scores of biscuits to determine the effect of recipe modifications versus increasing sucrose concentration and the corresponding Weibull model fits.

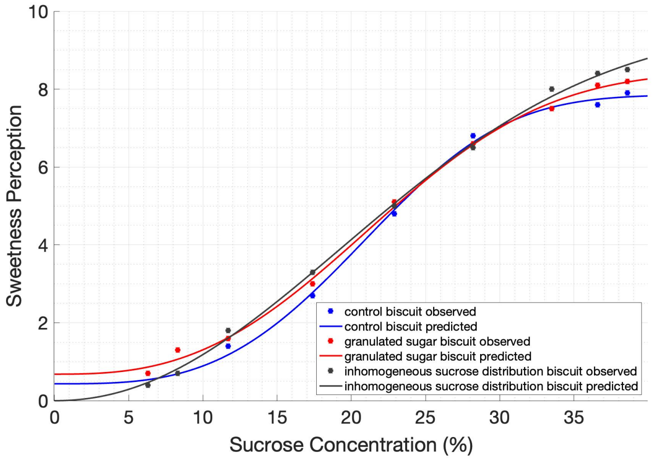


Figure 4.2. Sweetness perception scores of biscuits to determine the effect of the physical form of sucrose versus increasing sucrose concentration and the corresponding Weibull model fits.

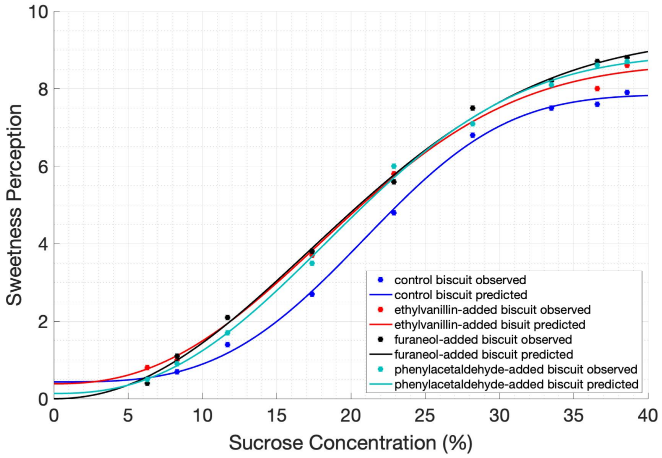


Figure 4.3. Sweetness perception scores of biscuits to determine the effect of the addition of aroma compounds versus increasing sucrose concentration and the corresponding Weibull model fits.

S_i is the lowest perceived sweetness given by the model and practically it can also be fixed to zero and it has only a minor effect on the predictive power of the model for semi-sweet biscuits. The reason for the large confidence intervals observed for S_i is that this value corresponds to zero sucrose concentration which is an extrapolating point of the model with the present data. The confidence intervals for S_i can be improved by collecting data near the lower asymptote when such low sweetness values (less than 6% in our observation) are needed to be assessed. It should be considered that the perception of sweetness diverges from the linearity at the lower and upper asymptotes of the model and therefore changing the sugar concentration around these levels has less significance for reformulation studies.

The upper asymptote of the modified Weibull model corresponds to S_m, the maximum perceived sweetness, and it can be defined where the intensity of perceived sweetness remains the same by increasing sugar concentration. All recipe modifications increased the S_m with respect to control (**Table 4.4**) but the statistically significant higher scores at the highest sucrose concentration (39%) were only observed for wholewheat, whey protein, pea protein, furaneol, and phenylacetaldehyde-containing recipes (**Table 4.1-3**).

A lower α value means that a desired sweetness perception level can be achieved at a lower sugar concentration. A particular decrease in the α parameter was observed for the biscuits with wholewheat with respect to control (**Table 4.4**). Shape parameter value β indicates that there is a lag in the increase of sugar perception despite increased sugar concentration. The β value was particularly higher for the control biscuit than all other recipes (**Table 4.4**). This showed that the recipe manipulations used in this study shortened the lag phase and increased the perceived sweetness at lower sucrose concentrations.

In the range of sucrose concentrations corresponding to the S_i and S_m values, the Weibull model allows finding the sucrose concentration that gives the desired perception of sweetness. For instance, to obtain the sweetness perception score of 4 (**Figure 4.1**) the required sucrose concentration in the recipe was 17% for wholewheat, 18% for whey protein-added, 21% for pea protein-added, 19% for control, and 20% for the low-fat biscuit.

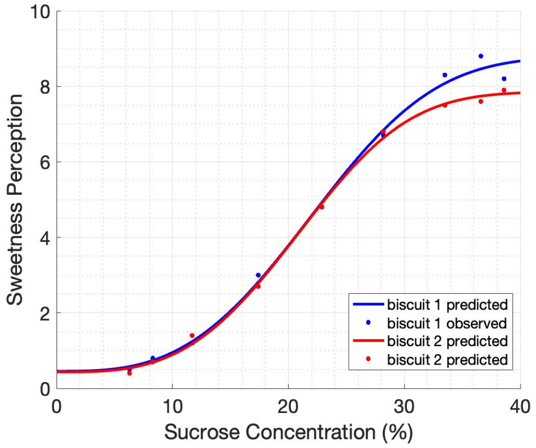


Figure 4.4. The sweetness perception scores of the control biscuits were obtained in two different panel settings versus increasing sucrose concentration and the corresponding Weibull model fits.

In **Figure 4.4**, the modified Weibull model applied to the sweetness scores obtained as a result of sensory analyzes performed both in the panel environment and in the special environments of the panellists in the same control biscuit containing 6-39% sugar is shown. The sigmoids obtained as a result of the two sensory analyzes show that the model fits the sweetness perception data very well.

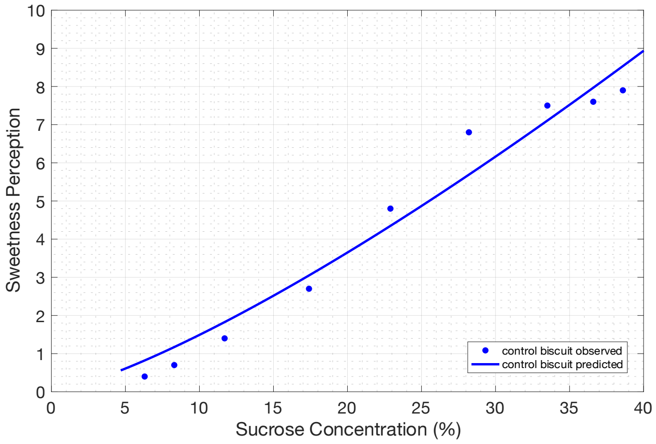


Figure 4.5. Sweetness perception scores of the control biscuits versus increasing sucrose concentration and the corresponding Power Law model fit.

On the other hand, when Steven's power law model was used to model the sweetness scores of the control biscuit corresponding to the sucrose concentrations used in the study, the model failed to determine the lower and upper limits of perceptible sweetness (**Figure 4.5**).

Table 4.4. Parameter estimates of the modified Weibull model with their relative 95% confidence intervals and the regression coefficients

Biscuit	S_i	S_m	β	α	**R^2**
Control biscuit	0.4 (±91%)	7.9 (±5%)	3.2 (±23%)	23.5 (±5%)	0.9982
Wholewheat biscuit	0.2 (±155%)	8.8 (±2%)	2.6 (±9%)	20.7 (±2%)	0.9997
Low fat biscuit	0	8.6 (±8%)	2.6 (±13%)	25.9 (±7%)	0.9986
Whey protein-added biscuit	0	9.2 (±15%)	2.0 (±22%)	23.7 (±17%)	0.9950
Pea protein-added biscuit	0	10.2 (±16%)	1.9 (±15%)	27.5 (±17%)	0.9980
Inhomogeneous distribution biscuit	0	9.7 (±11%)	2.1 (±13%)	26.5 (±11%)	0.9986
Granulated sugar biscuit	0.7 (±70%)	8.5 (±9%)	2.7 (±28%)	24.8 (±8%)	0.9980
Ethylvanillin-added biscuit	0.4 (±202%)	8.7 (±9%)	2.4 (±34%)	22.6 (±9%)	0.9973
Furaneol-added biscuit	0	9.4 (±8%)	2.1 (±13%)	23.4 (±9%)	0.9981
Phenylacetaldehyde-added biscuit	0.1 (±479%)	8.9 (±8%)	2.4 (±27%)	22.8 (±7%)	0.9982

Table 4.5. Flavour compounds of control, wholewheat, whey protein-added, and pea protein-added biscuits (ng/g)

Code	**LRI Observed**	**LRI literature**		**Control Biscuit**	**Wholewheat Biscuit**	**Whey Protein-Added Biscuit**	**Pea Protein-Added Biscuit**
			Alcohols				
AL1	1576	1576	2,3-Butanediol	1±0.2^{b}	1±0.2^{b}	1±0.1^{b}	2±0.3^{a}
AL2	1615	1610	(*E*)-2-Octen-1-ol	8±1^{a}	7±1^{a}	6±0.2^{a}	6±1^{a}
AL3	1877	1871	Benzenemethanol	285±16^{a}	281±15^{a}	208±18^{b}	285±35^{a}
AL4	1910	1919	2-Phenylethanol	195±27^{a}	166±45^{a}	218±27^{a}	241±33^{a}
			Aldehydes				
SA1	813	822	2-Methylpropanal	392±120^{b}	407±93^{a}	391±57^{a}	526±42^{a}
SA2	911	925	2(3)-Methylbutanal	1040±88^{c}	1708±432^{ab}	1451±186^{bc}	2245±117^{a}
SA3	1452	1454	Methional	10±2^{b}	15±3^{b}	13±2^{b}	20±2^{a}
SA4	1642	1678	Phenylacetaldehyde	92±14^{b}	176±54^{ab}	131±25^{ab}	232±68^{a}
A1	1078	1091	Hexanal	748±90^{b}	4033±646^{a}	666±80^{b}	409±66^{b}
A2	1389	1407	Nonanal	439±90^{a}	427±37^{a}	301±42^{b}	349±28^{ab}
A3	1424	1447	(*E*)-2-Octenal	18±2^{b}	64±11^{a}	17±3^{b}	23±3^{b}

A4	1531	1555	(*E*)-2-Nonenal	17±3^{b}	26±3^{a}	15±3^{b}	19±3^{ab}
A5	1764	1779	(*E,Z*)-Decadienal	19±3^{ab}	23±4^{a}	13±2^{b}	24±4^{a}
A6	1808	1814	(*E,E*)-2,4-Decadienal	64±12^{a}	65±7^{a}	37±4^{b}	67±11^{a}
			Aromatic Hydrocarbons				
H1	1519	1539	Benzaldehyde	572±49^{b}	749±40^{a}	590±51^{b}	784±62^{a}
H2	2016	2011	Methyleugenol	96±9^{b}	131±16^{a}	85±3^{b}	81±10^{b}
			Furan Derivatives				
F1	1226	1234	2-Pentylfuran	338±76^{b}	1247±226^{a}	273±55^{b}	522±75^{b}
F2	1464	1481	2-Furfural	688±139^{b}	1439±456^{a}	1278±215^{ab}	1011±139^{ab}
F3	1503	1503	2-Acetylfuran	187±22^{c}	291±50^{ab}	352±30^{a}	270±25^{b}
F4	1573	1574	5-Methyl-2-furfural	26±6^{a}	98±105^{a}	45±11^{a}	38±4^{a}
F5	1663	1665	2-Furanmethanol	476±47^{c}	1055±167^{a}	752±78^{b}	826±66^{b}
			Ketones				
K1	983	964	2,3-Butanedione	127±18^{a}	152±19^{a}	156±18^{a}	166±22^{a}
K2	1065	1065	2,3-Pentanedione	57±5^{b}	88±15^{a}	90±9^{a}	91±6^{a}
K3	1177	1164	2-Heptanone	85±16^{c}	527±75^{a}	92±16^{c}	345±36^{b}
K4	1279	1270	2-Octanone	1±0.1^{b}	2±0.1^{a}	1±0.1^{b}	2±0.1^{b}
K5	1295	1290	Acetol	47±6^{b}	67±8^{ab}	54±3^{b}	78±15^{a}

K6	1402	1408	3-Octen-2-one	19±2^{b}	88±12^{a}	17±2^{b}	27±3^{b}
K7	1567	1536	3,5-Octadien-2-one	21±2^{b}	66±5^{a}	18±1^{b}	61±8^{a}
			Lactones				
L1	1607	1603	γ-Valerolactone	1±0.1^{b}	2±0.2^{a}	1±0.1^{b}	1±0.1^{b}
L2	1626	1641	γ-Butyrolactone	29±4^{b}	50±8^{a}	36±3^{ab}	45±8^{a}
L3	1753	1758	γ-Crotonolactone	227±68^{b}	368±105^{ab}	418±63^{a}	507±93^{a}
L4	2076	2076	2-Nonenoic acid γ-lactone	132±17^{b}	1292±143^{a}	130±6^{b}	139±12^{b}
			Phenolic Compounds				
PC1	1861	1856	Guaiacol	47±5^{b}	170±17^{a}	67±4^{b}	64±2^{b}
PC2	2012	1997	Phenol	445±26^{a}	516±62^{a}	460±16^{a}	504±14^{a}
PC3	2201	2200	4-Vinyl-2-methoxyphenol	11±2^{b}	29±6^{a}	15±1^{b}	20±4^{b}
PC4	2402	2388	4-Vinylphenol	150±18^{b}	223±48^{ab}	232±18^{ab}	252±11^{a}
PC5	2569	2556	Vanillin	77±9^{b}	226±30^{a}	107±26^{b}	103±16^{b}
			Pyranones				
PN1	1965	1987	Maltol	7±2^{b}	17±2^{ab}	19±4^{a}	18±7^{a}
PN2	2270	2266	2,3-Dihidro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	2±2^{c}	5±3^{bc}	13±5^{ab}	15±7^{a}
			Pyrazines				
P1	1205	1223	Pyrazine	250±28^{b}	322±16^{a}	320±19^{a}	364±33^{a}

P2	1259	1278	2-Methylpyrazine	2698±291^{c}	3435±123^{ab}	3626±66^{a}	3012±272^{bc}
P3	1312	1327	2,5-Dimethylpyrazine	731±91^{ab}	561±58^{b}	684±85^{b}	923±121^{a}
P4	1319	1333	2,6-Dimethylpyrazine	729±38^{b}	938±57^{a}	923±60^{a}	943±74^{a}
P5	1326	1348	Ethylpyrazine	166±15^{b}	257±28^{a}	225±13^{a}	239±2^{a}
P6	1337	1340	2,3-Dimethylpyrazine	127±12^{b}	186±14^{a}	176±14^{a}	193±4^{a}
P7	1377	1393	2-Ethyl-6-methylpyrazine	69±7^{c}	136±11^{a}	100±10^{b}	85±5^{bc}
P8	1382	1398	2-Ethyl-5-methylpyrazine	54±9^{a}	62±8^{a}	52±4^{a}	67±6^{a}
P9	1394	1418	2-Ethyl-3-methylpyrazine	22±3^{c}	41±2^{a}	31±3^{b}	33±2^{b}
P10	1395	1399	Trimethylpyrazine	30±3^{c}	50±7^{b}	49±2^{b}	63±4^{a}
P11	1425	1437	2,6-Diethylpyrazine	2±0.3^{c}	5±1^{a}	3±0.2^{b}	3±0.4^{b}
P12	1431	1444	Ethenylpyrazine	82±10^{c}	114±9^{ab}	125±9^{a}	101±7^{bc}
P13	1436	1437	2-Ethyl-3,6-dimethylpyrazine	7±1^{c}	27±2^{a}	9±1^{c}	21±2^{b}
P14	1447	1469	2,3-Diethylpyrazine	0.4±0.1^{c}	1±0.1^{a}	1±0.02^{b}	1±0.1^{ab}
P15	1450	1552	2,5-Diethylpyrazine	1±0.1^{b}	1±0.1^{a}	1±0.02^{b}	1±0.1^{a}
P16	1453	1452	2-Ethyl-3,5-dimethylpyrazine	3±1^{c}	7±1^{a}	5±0.3^{b}	7±0.4^{a}
P17	1483	1492	2-Ethenyl-6-methylpyrazine	37±4^{b}	55±5^{a}	64±8^{a}	54±5^{a}
P18	1485	1485	2,3-Diethyl-5-methylpyrazine	301±35^{c}	1265±47^{a}	403±76^{c}	756±45^{b}
P19	1487	1499	2-Ethenyl-5(3)-methylpyrazine	12±2^{b}	18±2^{a}	18±2^{a}	17±1^{a}

P20	1505	1493	3,5(6)-Dimethyl-2-*n*-propylpyrazine	46±3^{d}	413±8^{a}	106±17^{c}	258±22^{b}
P21	1620	1627	2-Acetyl-3-methylpyrazine	1±0.1^{c}	2±0.1^{a}	1±0.1^{bc}	1±0.1^{ab}
P22	1623	1628	2-Acetylpyrazine	7±2^{b}	9±1^{b}	12±0^{a}	10±2^{ab}
P23	1679	1679	2-Acetyl-6-methylpyrazine	4±1^{c}	5±1^{bc}	7±0.4^{a}	6±1^{ab}
P24	1687	1704	2-Acetyl-5-methylpyrazine	4±1^{c}	6±1^{bc}	8±0.4^{a}	7±1^{ab}
P25	1989	2022	2-(2'-Furyl)-pyrazine	678±221^{a}	865±151^{a}	1090±359^{a}	728±65^{a}
			Pyrroles				
PR1	1517	1524	1*H*-Pyrrole	132±16^{c}	303±49^{a}	230±10^{b}	215±28^{b}
PR2	1714	1711	2-Ethyl-4-methyl-1*H*-pyrrole	13±2^{b}	39±7^{a}	29±7^{a}	40±5^{a}
PR3	1973	1969	2-Acetylpyrrole	54±10^{b}	93±6^{a}	87±8^{a}	80±6^{a}
PR4	2028	2036	1*H*-Pyrrole-2-carboxaldehyde	81±16^{c}	168±7^{ab}	119±9^{b}	98±10^{bc}
PR5	2109	2079	2-Formyl-5-methylpyrrole	7±2^{a}	10±1^{a}	11±1^{a}	9±0.1^{a}
			Sulfur Compounds				
S1	1067	1078	Dimethyl disulfide	10±1^{b}	12±1^{b}	106±14^{a}	17±4^{b}
S2	1369	1390	Dimethyl trisulfide	5±1^{b}	7±1^{b}	29±3^{a}	9±3^{b}
S3	1645	1653	2-Acetylthiazole	36±2^{ab}	42±3^{a}	35±4^{ab}	38±2^{b}
S4	1693	1684	2-Thiophenecarboxaldehyde	124±1^{b}	266±19^{a}	150±12^{b}	129±6^{b}

			Other Compounds				
PY	1598	1628	2-Acetylpyridine	59 ± 5^{b}	85 ± 12^{a}	75 ± 6^{ab}	80 ± 5^{a}
AC	1633	1647	Butanoic acid	8 ± 1^{a}	10 ± 2^{a}	9 ± 1^{a}	11 ± 1^{a}
CY	1828	1826	Cyclotene	176 ± 23^{a}	268 ± 39^{a}	196 ± 59^{a}	236 ± 21^{a}
FR	2037	2043	Furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone)	2 ± 1^{b}	5 ± 2^{ab}	9 ± 2^{a}	9 ± 3^{a}

* In a row, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

4.3. Changes in volatile compounds of biscuits by recipe modifications

The increase in sweetness perception in wholewheat or protein-added biscuits with respect to control directed us to investigate the changes in the volatile compounds found in the biscuits. The volatile compounds detected in the control, wholewheat, whey, and pea protein-added biscuits were given in **Table 4.5**. Alcohols (AL1-AL4), aldehydes (A1-A6) including Strecker aldehydes (SA1-SA4), aromatic hydrocarbons (H1, H2), furan derivatives (F1-F5), ketones (K1-K7), lactones (L1-L4), phenolic compounds (PC1-PC5), pyranones (PN1, PN2), pyrazines (P1-P25), pyrroles (PR1-PR5), sulphur compounds (S1-S4), and other compounds (PY, AC, CY, FR) were the compound groups detected in the biscuits, 77 individual compounds in total.

The PCA graph (**Figure 4.6**) shows the relationship of flavour compounds in control (C), wholewheat (WG), pea protein-added (PP), and whey protein-added (WP) biscuits. The first principal component (F1) contained 48.53% of the variance in the data and the second principal component (F2) contained 19.82%. Control and wholewheat biscuits were separated from the protein-added biscuits by the F1, while F2 differentiated the differences between control and wholewheat biscuits. Wholewheat biscuits were associated with an increase in pyrazines, aldehydes, ketones, alcohols, and several other compounds that impart caramel-like notes such as cyclotene, all of which might be related to increased perception of sweetness. The reason for this increase in wholewheat biscuits is the formation of more Maillard reaction products due to the higher amount of free amino acids in wholewheat flour compared to refined flour, and probably the formation of more lipid oxidation compounds because of unsaturated fatty acids found in wheat germ [73]. Similarly, the increase in Strecker aldehydes and some pyrazines in protein-added biscuits could be the reason for the increased sweetness, possibly due to the increase in these baked flavour compounds. As a result, wholewheat and protein-added biscuits contain higher amounts of compounds that give both caramel, roasted, and baked notes.

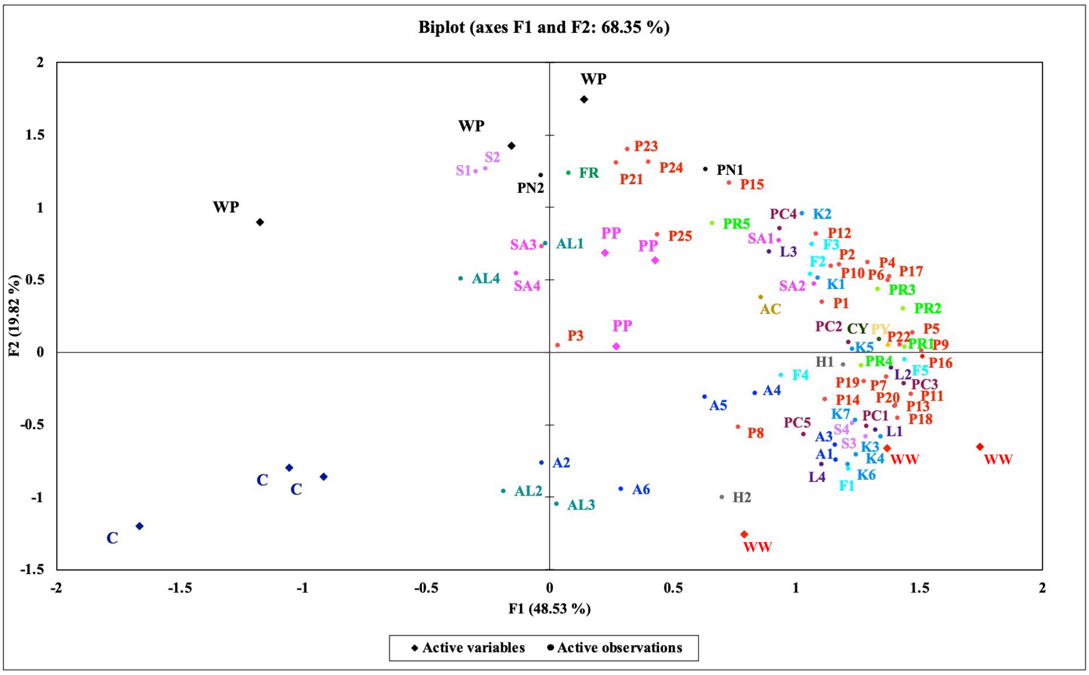


Figure 4.6. PCA biplots of control (C), wholewheat (WW), whey protein (WP), and pea protein (PP)-added biscuits as observations and aroma compounds as variables.

F1 and F2 are the first and second principal components, respectively. The list of volatile compound abbreviations is given in **Table 4.5**.

2/3-Methylbutanal and phenylacetaldehyde increased approximately 1-fold in wholewheat and protein-added biscuits (**Table 4.5**). 2-Methylbutanal and 3-methylbutanal have a malt or cocoa flavour, and phenylacetaldehyde has a floral and honey-like aroma which may be associated with an increased perception of sweetness. 2-Ethyl-3,6-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine, have relatively low odour thresholds and they have roasted, toasted, nutty, and earthy notes. About 3-fold increase was found in 2-ethyl-3,6-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine in wholewheat biscuits. Additionally, 2-ethyl-3,5-dimethylpyrazine doubled in wholewheat biscuits. These pyrazines together with Strecker aldehydes are key contributors to the baked flavour [66]. These pyrazines were also increased by protein addition to the biscuit. 4-Vinyl-2-methoxyphenol (sweet, spicy, and clove-like), guaiacol (smoke, spicy, vanilla, and woody), and vanillin (vanilla) are formed from the degradation of ferulic acid which is an abundant phenolic acid in wholewheat flours [42,74,75]. These compounds have also very low odour thresholds. Vanillin is well known to increase the sweetness perception. Other volatile compound groups that may affect the perception of sweetness were furanones and lactones. All lactones given in **Table 4.5** were higher in wholewheat biscuits compared with the control. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (caramel-like, burnt sugar, and candy floss) approximately doubled in wholewheat biscuits and increased by about 3-fold in protein-added biscuits. 2-Nonenoic acid γ-lactone (sweet, minty, and coconut) increased by 10-fold in wholewheat biscuits with respect to control and it is formed by oxidation of linoleic acid [76] which is an abundant fatty acid found in wheat germ. Therefore, these compounds were considered the reason for the increase in sweetness perception in wholewheat biscuits as they were in significantly higher concentrations than the control ($p<0.05$).

4.4. Changes in physical properties of biscuits

4.4.1. Effect of compositional changes

The hardness values of the biscuit doughs and biscuits containing varying sucrose concentrations (6-39%) were affected from the changes in composition (**Table 4.6** and **4.7**). It was found that only fat reduction affected the dough hardness among the changes made in the biscuit recipe. The hardness of low-fat biscuit doughs was higher than the hardness of control, wholewheat, whey, and pea protein-added biscuit doughs at all sugar concentrations (6-39%). The low-fat biscuits were not breakable under the force applied indicated that they had also the highest hardness values compared to control, wholegrain, whey, and pea protein biscuits. Maache-Rezzoug et al. [77] reported that as the amount of fat in the dough increased, the softness and homogeneity increased, which is in line with our data. However, increased hardness of low-fat biscuit dough (**Table 4.6**) and low-fat biscuits (**Table 4.7**) did not significantly change the sweetness perception of biscuits according to the results of sensory analysis (**Table 4.1**).The hardness of wholewheat biscuits was lower than control, low-fat, whey, and pea protein-added biscuits at sugar concentrations above the sugar concentrations of 17% (**Table 4.7**). Additionally, the hardness of dough of whole wheat biscuits was also lower than the hardness of other biscuit doughs (**Table 4.6**). This behaviour of dough could be explained by the interruption of the gluten network with fibers in the wholewheat. On contrary to our findings, Morales-Polanco et al. [78] reported that increasing the insoluble fibre content in biscuits prepared using wheat and chia flour increased the hardness of the biscuits, related to the fibre-gluten interaction. Agrahar-Murugkar et al. [79] reported the biscuit hardness was high in multi-nutrient biscuits produced using composite flours but they also reported that hardness of the dough of these biscuits was lower. Blanco Canalis et al. [67] reported that changing the fibre content had an effect on dough rheology and biscuit quality, but this effect depended on the type and amount of fibre added. Whey protein-added biscuits had higher hardness values compared to wholewheat biscuits at 8-23% sucrose concentrations. However, it was not possible to measure the hardness values of whey protein-added biscuits and pea protein-added biscuits at sucrose concentrations above 34% and 28%, respectively. Nogueira et al. [80] stated that adding a protein source weakens the biscuit dough structure and increases the biscuit hardness due to the competition of protein and sugar for water. The results obtained in our study show that the dough and hardness of low-fat biscuits are higher than control biscuits, but there is no difference ($p>0.05$) in the sweetness perception for these two biscuit types. However, wholewheat biscuits with lower dough and biscuit hardness were found to be sweeter than low-fat biscuits. Moreover, the sweetness perception changed for control biscuits and wholewheat biscuits, where there was no difference ($p>0.05$) between dough and biscuit hardness, and wholewheat biscuits were perceived as sweeter than control biscuits at sugar concentrations above 12%.

There was no significant differences ($p>0.05$) in the weight of the biscuits depending on the compositional changes except for the low-fat biscuits with 8 and 12% sucrose (**Table 4.8**). There was also no significant difference ($p>0.05$) between the spread ratio of the biscuits, except that wholewheat biscuits spread more than the others (**Table 4.9**). Therefore, it can be concluded that reducing fat or adding protein did not affect the spread ratio of biscuits.

Table 4.6. Hardness of biscuit dough with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	12.8 ± 0.5^{cd}	9.6 ± 0.7^{bc}	8.6 ± 0.4^{bc}	9.0 ± 0.6^{b}	8.0 ± 1.1^{b}	8.3 ± 0.7^{b}	9.2 ± 0.1^{b}	11.9 ± 0.8^{b}	11.8 ± 0.8^{b}
Wholewheat biscuit	8.8 ± 1.4^{d}	6.7 ± 0.4^{c}	5.4 ± 1.1^{c}	2.8 ± 0.2^{c}	3.2 ± 1.0^{b}	4.4 ± 0.9^{a}	4.6 ± 0.9^{c}	5.2 ± 1.6^{b}	6.6 ± 1.6^{b}
Low fat biscuit	26.8 ± 1.4^{a}	27.0 ± 2.8^{a}	26.4 ± 1.7^{a}	21.4 ± 2.9^{a}	20.1 ± 3.1^{a}	20.2 ± 0.9^{a}	29.8 ± 2.3^{a}	34.5 ± 6.0^{a}	40.6 ± 6.2^{a}
Whey protein-added biscuit	16.4 ± 0.3^{bc}	12.5 ± 3.6^{bc}	12.4 ± 4.2^{b}	5.4 ± 0.5^{bc}	6.7 ± 0.5^{b}	7.1 ± 0.7^{b}	10.0 ± 0.5^{b}	10.4 ± 0.7^{b}	13.9 ± 0.1^{b}
Pea protein-added biscuit	18.7 ± 4.0^{b}	15.5 ± 0.6^{b}	9.3 ± 0.5^{bc}	6.3 ± 0.5^{bc}	5.4 ± 0.1^{b}	7.0 ± 0.2^{b}	11.5 ± 1.2^{b}	12.4 ± 0.4^{b}	14.4 ± 0.4^{b}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.7. Hardness of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	18.1 ± 4.7^{a}	18.6 ± 4.2^{bc}	20.6 ± 1.4^{b}	45.4 ± 4^{a}	39.8 ± 3.1^{a}	43.3 ± 4.9^{a}	37.9 ± 2.3	49.5 ± 0.5	33.1 ± 5.6
Wholewheat biscuit	11.4 ± 1.2^{a}	8.8 ± 1.5^{c}	13.9 ± 0.2^{b}	12.3 ± 2.6^{b}	27.5 ± 2.9^{b}	23.6 ± 0.5^{b}	37.5 ± 2.8	20.9 ± 0.5	38.5 ± 3.2
Whey protein-added biscuit	14.9 ± 3.1^{a}	32.0 ± 0.1^{a}	38.0 ± 5.0^{a}	33.6 ± 11^{a}	46.5 ± 1.9^{a}	36.8 ± 8.0^{ab}	-	-	-
Pea protein-added biscuit	15.7 ± 3.2^{a}	24.3 ± 2.4^{b}	23.3 ± 3.6^{b}	32.7 ± 0.8^{a}	40.0 ± 4.1^{a}	-	-	-	-

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.8. Weight of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	8.9 ± 0.1^{ab}	9.2 ± 0.1^{bc}	9.2 ± 0.2^{b}	9.4 ± 0.3^{ab}	9.5 ± 0.2^{b}	10.0 ± 0.4^{a}	9.7 ± 0.1^{a}	10.0 ± 0.2^{b}	10.1 ± 0.3^{a}
Wholewheat biscuit	8.9 ± 0.2^{ab}	8.9 ± 0.2^{c}	8.8 ± 0.2^{b}	9.0 ± 0.1^{b}	8.9 ± 0.2^{b}	9.2 ± 0.3^{a}	9.6 ± 0.4^{a}	9.4 ± 0.5^{b}	9.4 ± 0.2^{a}
Low fat biscuit	9.3 ± 0.3^{ab}	10.0 ± 0.1^{a}	10.3 ± 0.5^{a}	9.9 ± 0.3^{ab}	10.6 ± 0.2^{a}	9.7 ± 0.2^{a}	9.4 ± 0.4^{a}	9.3 ± 0.3^{b}	9.6 ± 0.3^{a}
Whey protein-added biscuit	8.8 ± 0.3^{b}	10.1 ± 0.5^{ab}	10.3 ± 0.1^{ab}	9.4 ± 0.4^{ab}	10.0± 0.5^{ab}	10.1 ± 0.4^{a}	10.1 ± 0.4^{a}	11.1 ± 0.8^{a}	9.9 ± 0.5^{a}
Pea protein-added biscuit	9.5 ± 0.2^{a}	9.9 ± 0.2^{ab}	9.5 ± 0.1^{ab}	9.6 ± 0.3^{ab}	9.9 ± 0.7^{ab}	9.9 ± 0.1^{a}	9.8 ± 0.6^{a}	9.8 ± 0.4^{b}	9.3 ± 0.6^{a}

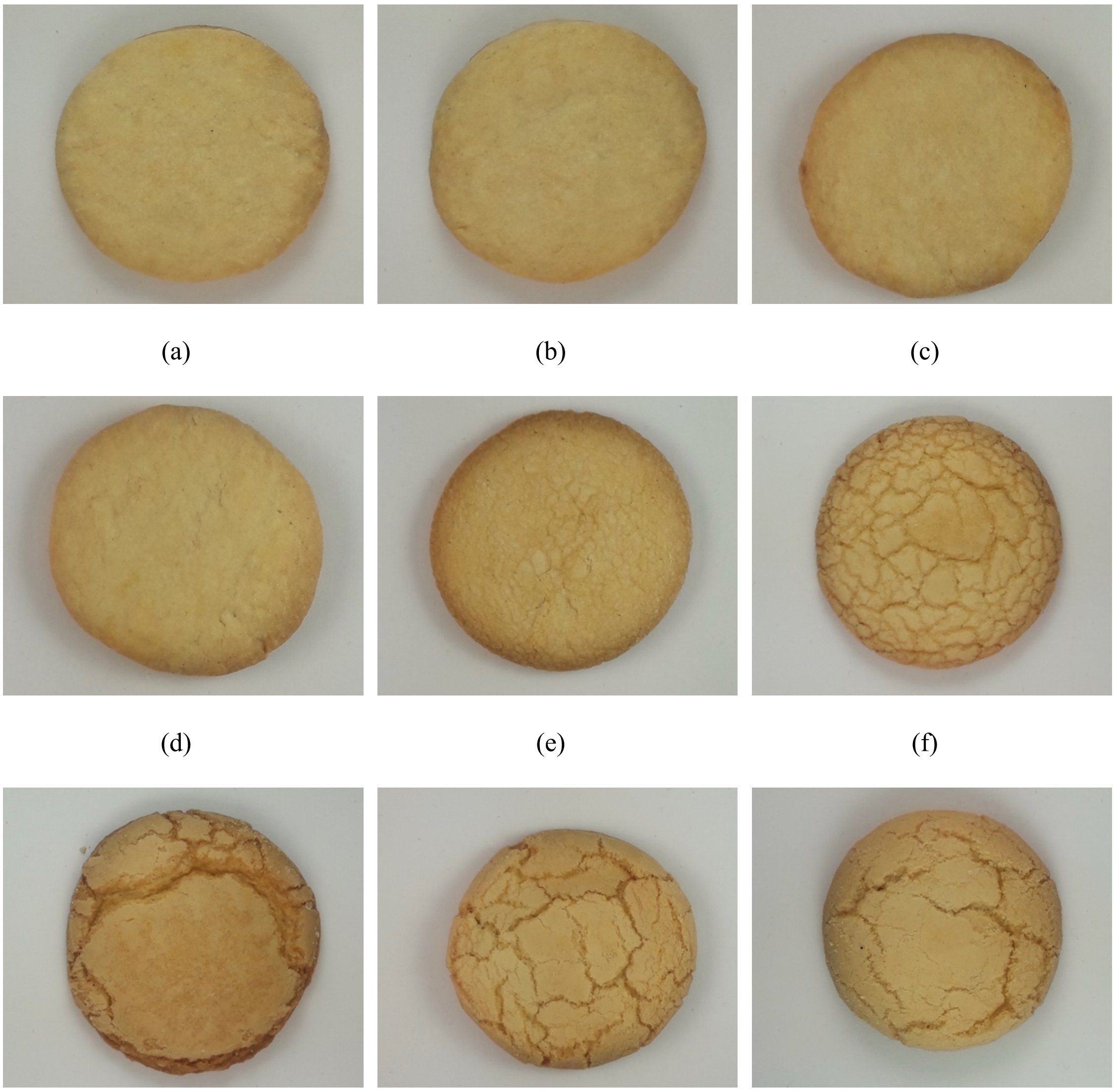
* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.9. Spread ratio of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	4.6 ± 0.3^{b}	4.8 ± 0.1^{b}	4.8 ± 0.2^{b}	6.3 ± 0.3^{c}	5.7 ± 0.2^{b}	6.8 ± 0.2^{ab}	7.0 ± 0.1^{a}	5.4 ± 1.0^{a}	4.0 ± 0.3^{a}
Wholewheat biscuit	5.9 ± 0.3^{a}	5.8 ± 0.1^{a}	5.8 ± 0.1^{a}	6.2 ± 0.1^{a}	7.2 ± 0.2^{a}	7.6 ± 0.6^{a}	5.7 ± 0.3^{a}	3.3 ± 0.5^{a}	2.7 ± 0.1^{b}
Low fat biscuit	4.8 ± 0.3^{b}	4.1 ± 0.1^{c}	4.7 ± 0.5^{b}	5.1 ± 0.1^{bc}	4.7 ± 0.3^{c}	5.1 ± 0.1^{c}	6.1 ± 0.3^{a}	3.6 ± 0.9^{a}	2.7 ± 0.2^{bc}
Whey protein-added biscuit	5.2 ± 0.1^{ab}	4.1 ± 0.1^{c}	4.4 ± 0.3^{b}	5.7 ± 0.1^{ab}	5.6 ± 0.1^{b}	6.4 ± 0.1^{b}	6.6 ± 1.0^{a}	3.9 ± 0.3^{a}	3.2 ± 0.1^{bc}
Pea protein-added biscuit	4.9 ± 0.5^{b}	4.8 ± 0.3^{b}	5.0 ± 0.3^{ab}	5.5 ± 0.3^{b}	5.4 ± 0.2^{b}	6.3 ± 0.2^{b}	6.2 ± 0.5^{a}	3.3 ± 0.2^{a}	3.2 ± 0.1^{b}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Similarly, in the study of Aggarwal et al. [81], the spread ratios, and hardness values of biscuits increased according to fibre content. Sozer et al. [82] stated that when more than 5% wheat bran was added to the biscuits, the weight, diameter, height, and spreading speed of the biscuits increased.

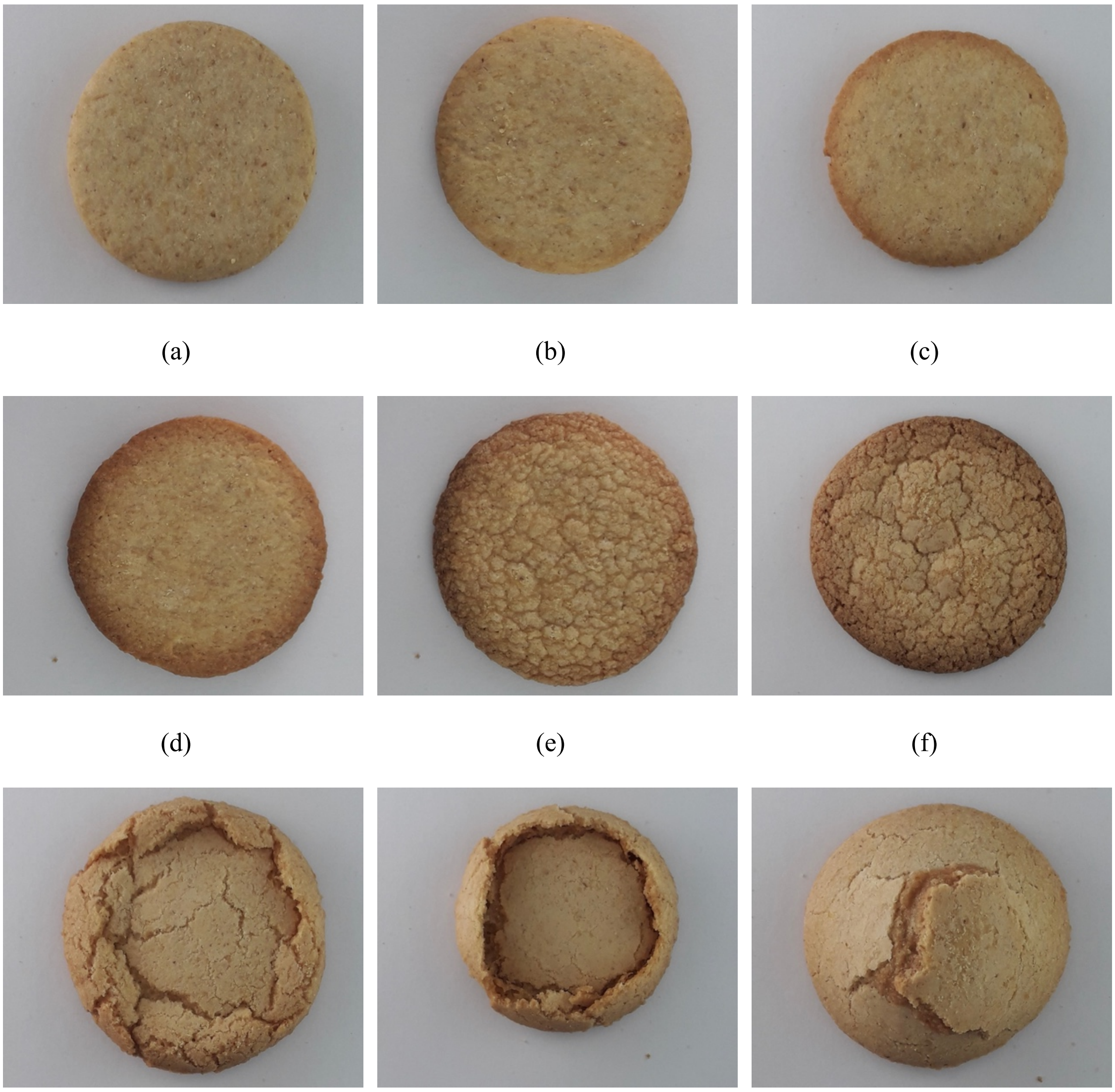


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.7. Example of control biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

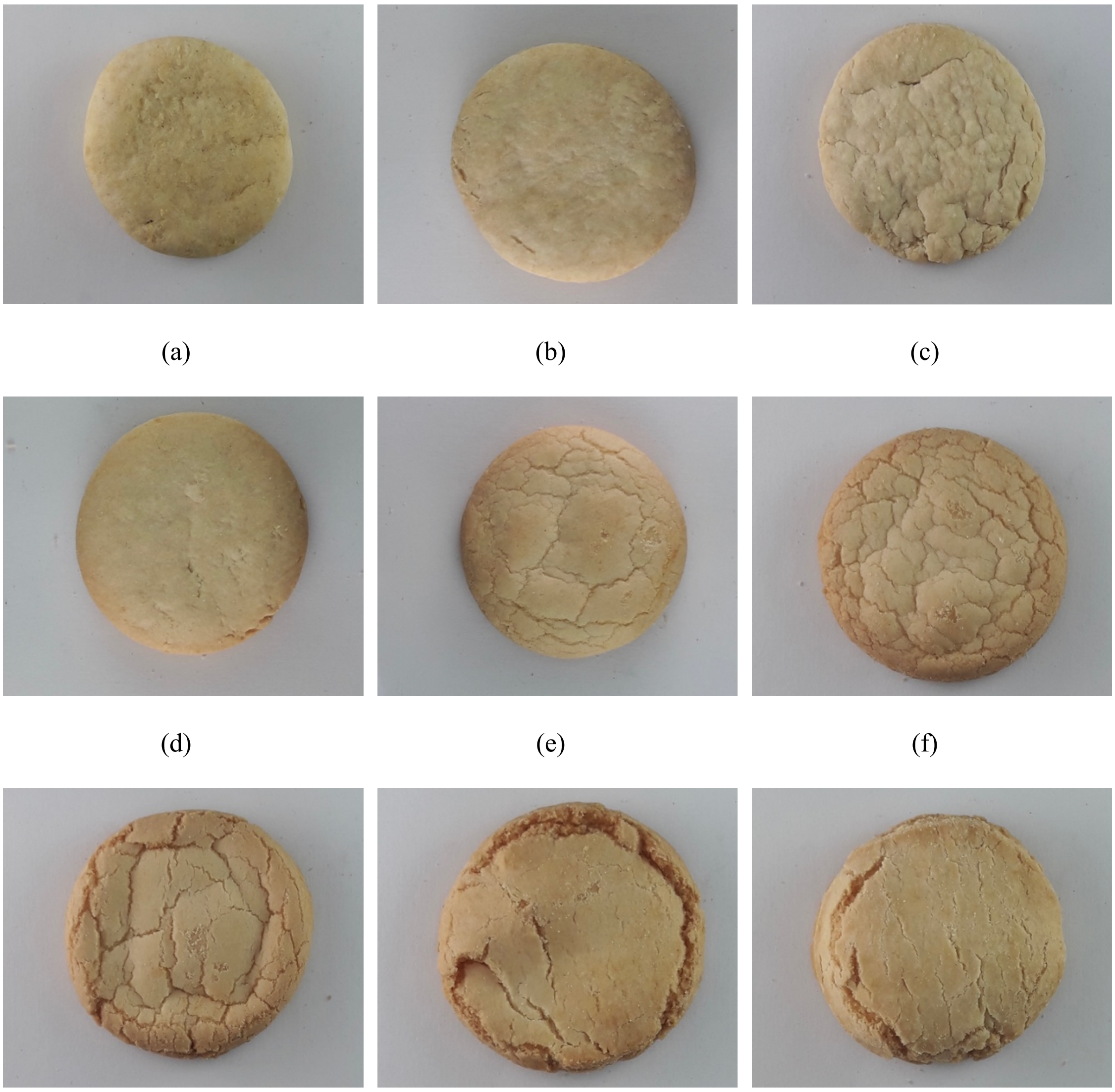


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.8. Example of wholewheat biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

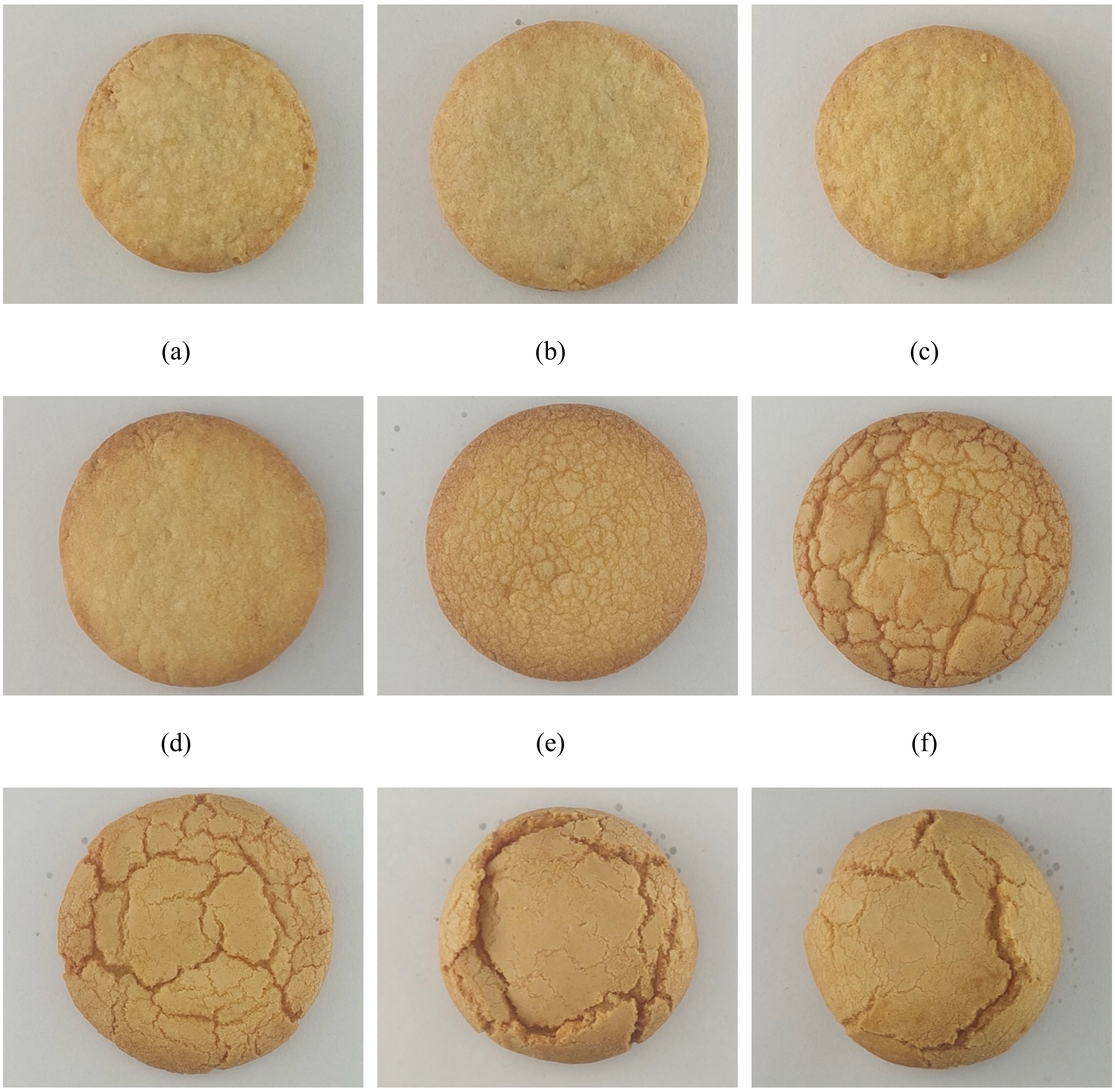


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.9. Example of low-fat biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

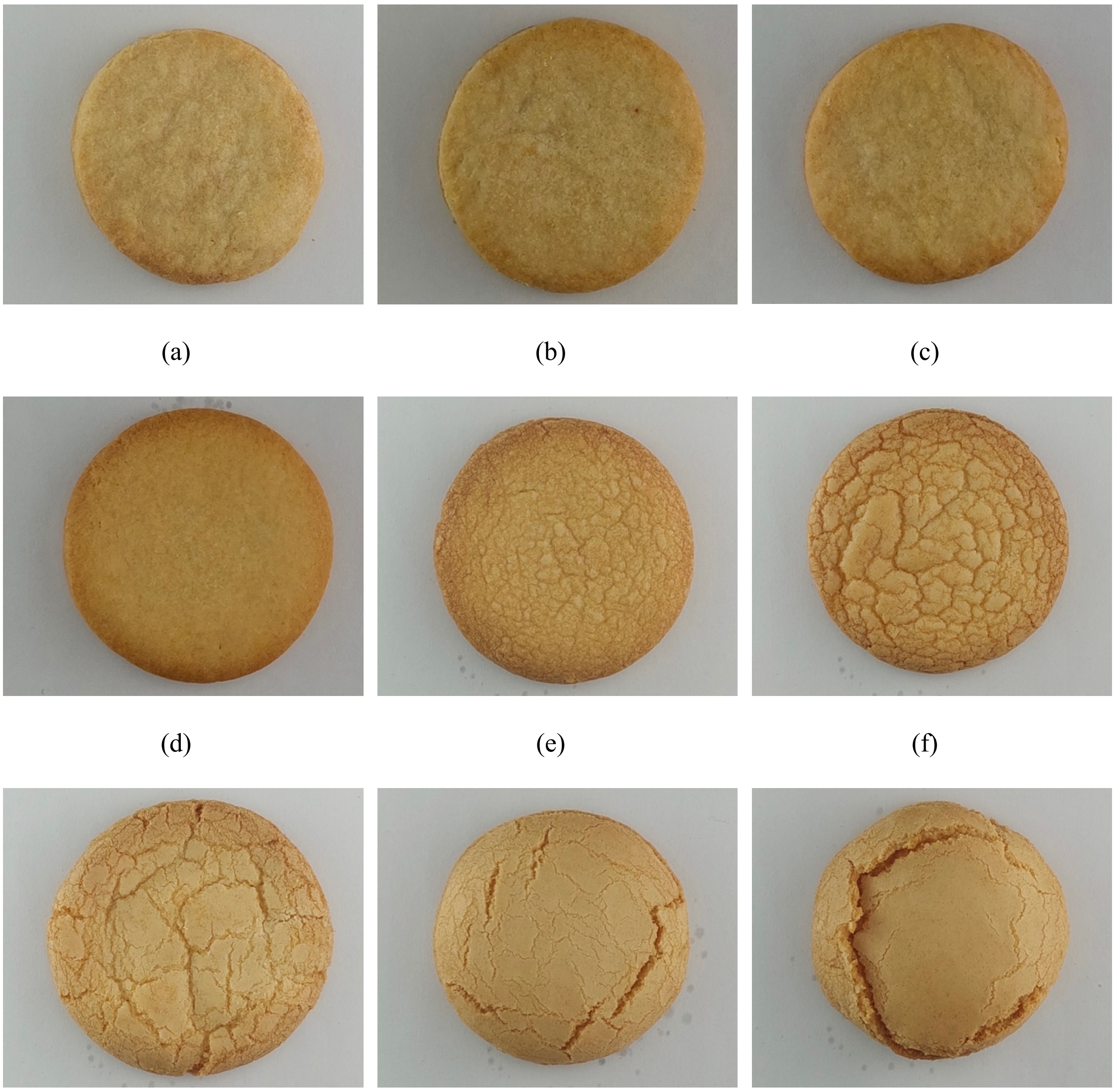


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.10. Example of whey protein-added biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.11. Example of pea protein-added biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

As a result of caramelization and the Maillard reaction occurs during baking process, browning occurs starting from the edges of the biscuits and growing towards the centre, resulting in a colour gradient on the surface [64]. This colour formation is expressed by the browning ratio and it is known that as a result of these reactions, taste and odour-active compounds that affect the sweetness intensity are formed.

L*, a*, and b* values and the browning ratio of biscuits were determined by using the images of the biscuits and examples of these images was shown in **Figures 4.7-11**. L* values of biscuits are shown in **Table 4.10**. Whey protein-added biscuits had higher L* values compared to wholewheat biscuits at 6-37% sucrose concentrations, and higher than control at 12, 23, and 28% sucrose concentrations. At 6-34% of sucrose, protein-added biscuits had higher a* values compared to control biscuits (**Table 4.11**). b* values are shown in **Table 4.12**, and protein-added biscuits had higher b* values at each sucrose concentration compared to others. L* and b* value of wholewheat biscuits mostly decreased significantly in varying sucrose contents compared to control while a* value mostly increased ($p<0.05$). There was no significant difference ($p>0.05$) founded in browning ratios of biscuits (**Table 4.13)**.

Nogueira et al. [80] reported that increasing the protein content increased a* values, and caused darker coloured biscuits which was due to the increase in the number of amino groups participating the Maillard reaction with protein enrichment. In addition to these findings, which are compatible with our results, the fact that protein enrichment using whey protein is more effective on colour than the addition of pea protein has been explained by the lactose content of whey protein [83]. Sozer et al. [82] reported that the addition of wheat bran increased the L* value and decreased the b* value in biscuits. However, in the study of Agrahar-Murugkar et al. [79], while the use of composite flour increased the L* value of the biscuits, it did not affect the a* and b* values. It was interpreted that the differences between the results of those aforementioned studies and this study were due to the use of different fibre source.

Table 4.10. L* value of biscuit with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	62.5 ± 0.2^{ab}	61.7 ± 0.3^{ab}	61.4 ± 1.6^{b}	61.4 ± 0.2^{b}	58.6 ± 0.4^{a}	58.4 ± 0.8^{b}	55.5 ± 2.1^{a}	57.6 ± 1.2^{a}	59.7 ± 2.1^{a}
Wholewheat biscuit	51.7 ± 0.6^{b}	52.6 ± 0.5^{b}	51.7 ± 1.1^{c}	48.9 ± 0.1^{b}	46.9 ± 0.6^{c}	46.7 ± 1.9^{b}	48.7 ± 1.3^{c}	48.1 ± 5.1^{b}	52.6 ± 0.5^{a}
Low fat biscuit	57.7 ± 0.9^{ab}	59.2 ± 0.2^{ab}	60.5 ± 0.6^{b}	58.8 ± 0.3^{ab}	58.6 ± 0.4^{b}	56.6 ± 0.1^{a}	55.1 ± 0.3^{b}	56 ± 0.5^{ab}	56.3 ± 2.5^{a}
Whey protein-added biscuit	66.6 ± 0.3^{a}	66.1 ± 0.5^{a}	68.4 ± 1.1^{a}	61.3 ± 3.4^{a}	64.6 ± 2.3^{a}	61.2 ± 0.9^{a}	61.4 ± 1.8^{a}	63.9 ± 0.1^{a}	60.7 ± 0.8^{a}
Pea protein-added biscuit	56.2 ± 6.3^{ab}	55.9 ± 5.9^{ab}	52.5 ± 0.7^{c}	56.2 ± 5.1^{ab}	58.6 ± 0.7^{b}	56.5 ± 1.9^{a}	63 ± 0.7^{a}	60.3 ± 0.3^{a}	60.3 ± 3.9^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05

Table 4.11. a* value of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	2.7 ± 0.4^{b}	3.4 ± 0.3^{b}	3.6 ± 0.9^{b}	3.7 ± 0.7^{b}	5.9 ± 0.3^{b}	6.7 ± 0.1^{c}	8.6 ± 0.3^{c}	7.8 ± 0.5^{ab}	5.6 ± 0.8^{b}
Wholewheat biscuit	5.2 ± 0.3^{a}	6.1 ± 0.3^{a}	7 ± 0.3^{b}	10.1 ± 0.2^{a}	10.7 ± 0.3^{a}	10.3 ± 0.2^{b}	9.6 ± 0.1^{b}	8.8 ± 1.9^{ab}	7.1 ± 0.5^{b}
Low fat biscuit	2.5 ± 0.1^{b}	3.2 ± 0.7^{b}	2.3 ± 0.1^{b}	3.6 ± 0.5^{b}	4.4 ± 0.1^{b}	5.9 ± 0.4^{c}	6.6 ± 0.3^{d}	6.7 ± 0.1^{b}	5.6 ± 2.1^{b}
Whey protein-added biscuit	5.3 ± 0.9^{a}	6.0 ± 0.3^{a}	6.4 ± 1.9^{b}	9.9 ± 2.4^{a}	10.5 ± 0.8^{a}	12.5 ± 0.2^{a}	11.5 ± 0.1^{a}	10.6 ± 0.2^{a}	10.9 ± 1.4^{a}
Pea protein-added biscuit	6.5 ± 0.2^{a}	7.1 ± 0.5^{a}	9.9 ± 2.4^{a}	10.6 ± 1.6^{a}	11.2 ± 0.5^{a}	12.9 ± 0.7^{a}	9.5 ± 0.2^{b}	8.9 ± 1.1^{ab}	9.6 ± 0.1^{ab}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05

Table 4.12. b* value of biscuit with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	30.8 ± 1.8^{b}	29.5 ± 1.6^{b}	29.4 ± 0.9^{b}	30.5 ± 1.1^{c}	31.1 ± 1.4^{bc}	31.2 ± 0.9^{c}	30.2 ± 1.2^{b}	31.9 ± 0.8^{c}	29.1 ± 0.6^{b}
Wholewheat biscuit	26.2 ± 0.2^{c}	28.4 ± 0.3^{b}	27.0 ± 0.5^{b}	28.1 ± 0.1^{c}	26.8 ± 1.1^{c}	25.5 ± 1.8^{cd}	26.1 ± 1.3^{c}	25.2 ± 0.3^{d}	25.2 ± 1.2^{c}
Low fat biscuit	23.5 ± 0.3^{c}	18.8 ± 0.7^{c}	17.7 ± 0.8^{c}	19.8 ± 0.4^{d}	19.3 ± 0.1^{d}	20.0 ± 0.3^{d}	20.3 ± 0.3^{d}	20.4 ± 0.8^{e}	18.8 ± 0.3^{d}
Whey protein-added biscuit	38.2 ± 0.2^{a}	33.2 ± 3.8^{ab}	37.5 ± 4.2^{a}	38.0 ± 1.7^{b}	34.5 ± 3.0^{b}	38.2 ± 0.5^{b}	37.9 ± 0.9^{a}	40.8 ± 0.6^{a}	39.0 ± 1.4^{a}
Pea protein-added biscuit	38.4 ± 0.1^{a}	40.1 ± 1.9^{a}	41.3 ± 0.5^{a}	44.0 ± 1.5^{a}	42.5 ± 0.5^{a}	44.4 ± 2.5^{a}	39.6 ± 0.4^{a}	37.1 ± 1.1^{b}	38.5 ± 0.6^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05

Table 4.13. Browning ratio of biscuits with recipe modifications*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{a}	0.3 ± 0.1^{ab}	0.5 ± 0.1^{b}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.6 ± 0.1^{abc}
Wholewheat biscuit	0.2 ± 0.2^{a}	0.2 ± 0.1^{a}	0.1 ± 0.1^{c}	0.2 ± 0.1^{a}	0.2 ± 0.1^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{a}	0.4 ± 0.2^{a}	0.8 ± 0.1^{a}
Low fat biscuit	0.5 ± 0.3^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.1^{a}	0.6 ± 0.2^{a}	0.3 ± 0.1^{a}	0.6 ± 0.2^{ab}
Whey protein-added biscuit	0.4 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{bc}	0.3 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.4 ± 0.1^{b}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.1^{bc}
Pea protein-added biscuit	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{bc}	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.4 ± 0.1^{b}	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{bc}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05

4.4.2. Effect of physical characteristics of sucrose

The hardness values of biscuit doughs and biscuits containing different forms of sucrose are given in **Table 4.14 and 4.15**. Presence of granulated sugar caused decrease in the hardness values of the dough. The reason for that may be the water holding capacity of granulated sugar compared to powdered sugar. Drastic decreases in the hardness values of doughs with sugar concentrations higher than 34% was observed where hardness of control biscuit > inhomogeneous sucrose distribution biscuit > granulated sugar biscuit. However, inhomogeneous distribution biscuits were harder than granulated sugar biscuits and control because it was impossible to break inhomogeneous biscuits under the force applied. This behaviour of the inhomogeneous distribution of sucrose in biscuits could be attributed to insufficient creaming due to inhomogeneous distribution of sucrose.

There was no significant difference ($p>0.05$) between the weight of the biscuits containing different sucrose forms (**Table 4.16**). Spread ratios of the biscuits with different sucrose forms were given in **Table 4.17**. In general there was no statistically significant differences ($p>0.05$) in spread ratio depending on the form of sugar except for the inhomogeneous distribution biscuits having %28 and 37% sucrose.

Molina et al. [68] observed that the thickness of the biscuits with granulated sugar was higher than the biscuits with powder sugar. Tyuftin et al. [6], on the other hand, reported that low sugar granule size reduced the diameter and length of biscuits, but it did not affect the height. The fact that the mentioned difference was not observed in our results was attributed to the biscuit type and the different granule sizes of the sugar used.

Table 4.14. Hardness of biscuit dough with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	12.8 ±0.5^{a}	9.6 ± 0.7^{b}	8.6 ± 0.4^{a}	9.0 ± 0.6^{b}	8.0 ± 1.1^{a}	8.3 ± 0.7^{b}	9.2 ± 0.1^{b}	11.9 ±0.8^{a}	11.8 ±0.8^{a}
Inhomogeneous sucrose distribution biscuit	22.3 ± 6.6^{a}	22.4 ±1.2^{a}	16.1 ±4.8^{a}	13.1 ±1.3^{a}	13.2 ± 2.0^{a}	14.4 ± 5.7^{a}	14.8 ±1.1^{a}	8.5 ± 0.9^{b}	8.7 ± 0.6^{b}
Granulated sugar biscuit	19.4 ±1.2^{a}	13 ± 2.7^{b}	11.5 ±6.6^{a}	10.9 ±2.6^{a}	5.8 ± 0.9^{b}	5.2 ± 0.2^{a}	4.5 ± 0.2^{c}	5.6 ± 0.3^{c}	5.5 ± 0.5^{c}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.15. Hardness of biscuits with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	18.1 ± 4.7	18.6 ± 4.2	20.6 ± 1.4	45.4 ± 4	39.8 ± 3.1	43.3 ± 4.9	37.9 ± 2.3	49.5 ± 0.5	33.1 ± 5.6
Granulated sugar biscuit	12.9 ± 2.6	18.7 ± 1.9	22.4 ± 1.6	30 ± 2.7	43.6 ± 2.5	47.6 ± 7.9	45.3 ± 0.5	43.3 ± 0.3	46.4 ± 3.4

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.16. Weight of biscuits with different forms of sucrose*

Biscuit	Sugar content (%)								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	8.9 ± 0.1^{a}	9.2 ± 0.1^{a}	9.2 ± 0.2^{a}	9.4 ± 0.3^{a}	9.5 ± 0.2^{b}	10.0 ± 0.4^{a}	9.7 ± 0.1^{b}	10.0 ± 0.2^{a}	10.1 ± 0.3^{a}
Inhomogeneous sucrose distribution biscuit	9.4 ± 0.3^{a}	8.7 ± 0.2^{b}	9.2 ± 0.4^{a}	10.1 ± 0.5^{a}	10.2 ± 0.1^{a}	9.6 ± 0.4^{a}	10.3 ± 0.3^{a}	10.1 ± 0.1^{a}	10.2 ± 0.1^{a}
Granulated sugar biscuit	9.6 ± 0.2^{a}	9.5 ± 0.1^{a}	9.6 ± 0.2^{a}	9.9 ± 0.7^{a}	9.8 ± 0.2^{ab}	10.1 ± 0.1^{a}	10 ± 0.1^{ab}	9.9 ± 0.1^{a}	10.5 ± 0.3^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.17. Spread ratio of biscuits with different forms of sucrose*

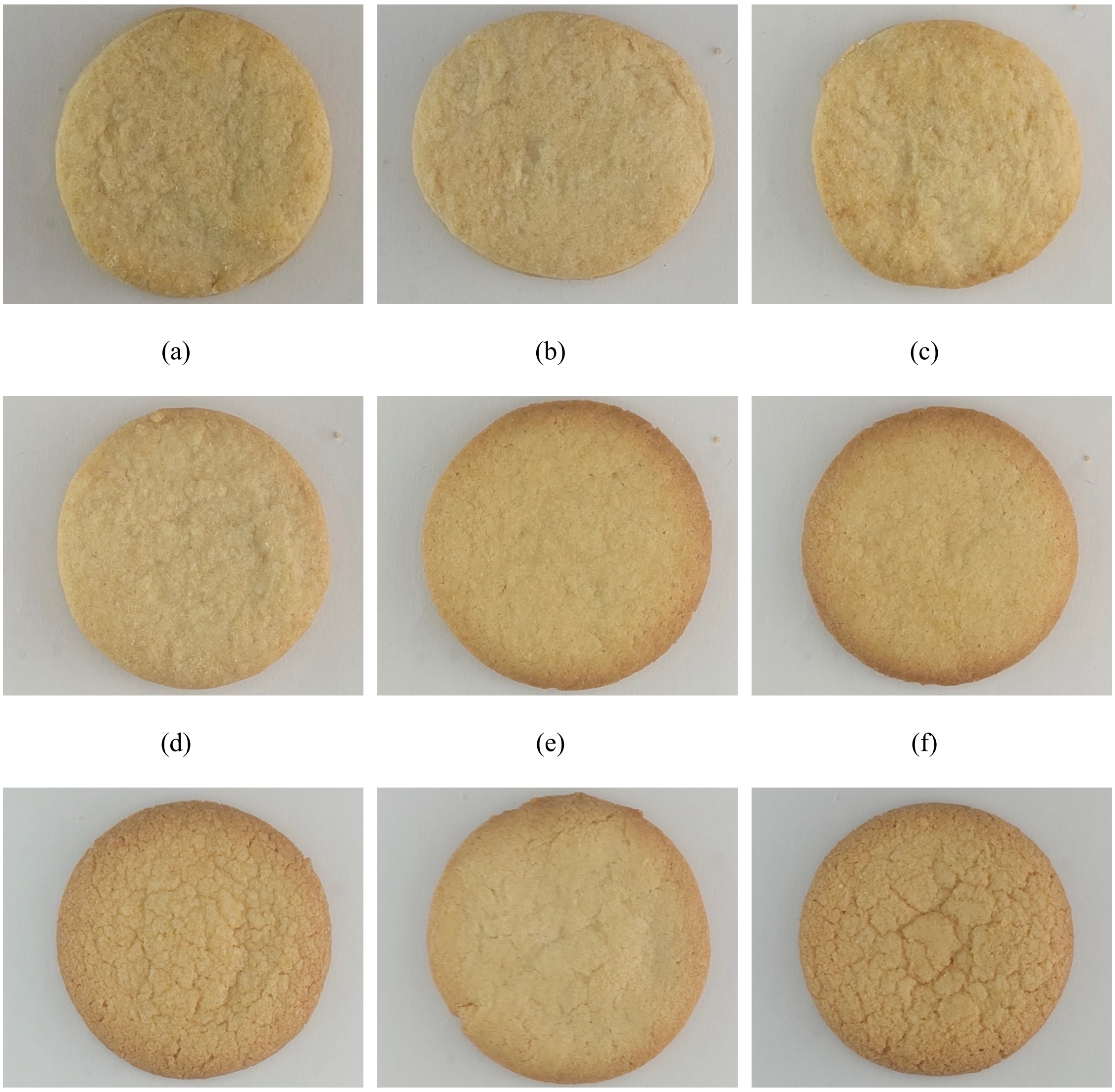
Biscuit	Sugar content (%)								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	4.6 ± 0.3^{a}	4.8 ± 0.1^{a}	4.8 ± 0.2^{a}	6.3 ± 0.3^{a}	5.7 ± 0.2^{ab}	6.8 ± 0.2^{a}	7.0 ± 0.1^{a}	5.4 ± 1.0^{a}	4.0 ± 0.3^{b}
Inhomogeneous sucrose distribution biscuit	4.7 ± 0.4^{a}	4.7 ± 0.5^{a}	4.6 ± 0.1^{a}	4.4 ± 0.3^{a}	5.0 ± 0.5^{b}	5.4 ± 0.5^{b}	6.5 ± 0.2^{b}	7.4 ± 0.5^{a}	7.5 ± 0.2^{a}
Granulated sugar biscuit	4.6 ± 0.4^{a}	4.1 ± 0.1^{a}	4.5 ± 0.3^{a}	5.2 ± 0.5^{a}	6.2 ± 0.1^{a}	6.6 ± 0.3^{a}	7.0 ± 0.2^{a}	7.0 ± 0.1^{a}	7.0 ± 0.1^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

L*, a*, and b* values and the browning ratio of biscuits containing different forms of sucrose was determined by using the biscuit images and examples of these images were given in **Figure 4.7**, **Figure 4.12**, **Figure 4.13**, and L*, a*, b* values were given in **Table 4.18-21, respectively**.

At 12-34% sucrose levels, control biscuits had lower L* values than granulated sugar biscuits (**Table 4.18**). Inhomogeneous sucrose distribution biscuits had higher a* values than other biscuits above 23% sucrose concentrations (**Table 4.19**). They had also higher b* values than other biscuits above 6% sucrose concentrations (**Table 4.20**). The browning ratio of inhomogeneous sucrose distribution biscuits was higher than granulated sugar biscuits at 23 and 28% of sucrose levels, and control biscuits at 23% of sucrose (**Table 4.21**).

Tyuftin et al. [6], on the other hand, observed a darker colour after baking when finely ground sugar was used in the biscuit formulation. The fact that this result did not overlap with the results we obtained in our study could be that the colour was determined by sensory analysis in the study of Tyuftin et al. [6] and the size of the sugar was different than the size of sugar in our study.

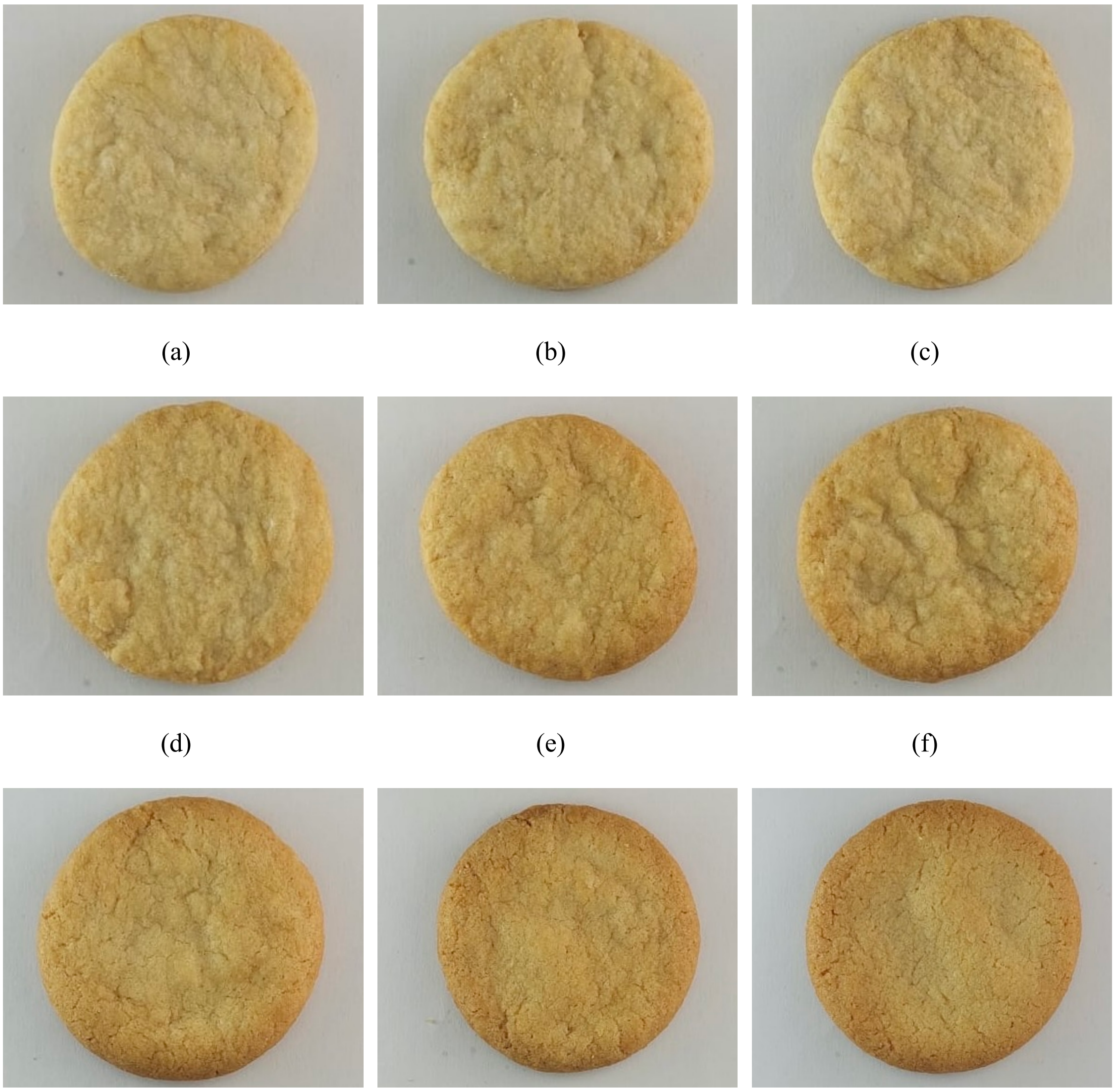


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.12. Example of granuleted sugar biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.13. Example of inhomogeneous sucrose distribution biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

Table 4.18. L* value of biscuits with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	62.5 ± 0.2^{b}	61.7 ± 0.3^{a}	61.4 ± 1.6^{b}	61.4 ± 0.2^{c}	58.6 ± 0.4^{b}	58.4 ± 0.8^{b}	55.5 ± 2.1^{c}	57.6 ± 1.2^{a}	59.7 ± 2.1^{a}
Inhomogeneous sucrose distribution biscuit	67.2 ± 0.6^{a}	62.7 ± 4.4^{a}	66.5 ± 2.9^{ab}	64.1 ± 0.8^{b}	63.3 ± 0.8^{a}	61.6 ± 0.5^{b}	61.6 ± 1.4^{ab}	60.6 ± 0.1^{b}	60.7 ± 0.9^{a}
Granulated sugar biscuit	61.6 ± 0.1^{b}	67.6 ± 2.0^{a}	67.7 ± 1.8^{a}	68.6 ± 1.0^{a}	65.1 ± 3.2^{a}	66.6 ± 0.6^{a}	65.3 ± 0.2^{a}	67.6 ± 0.2^{a}	64.3 ± 3.9^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.19. a* value of biscuits with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	2.7 ± 0.4^{a}	3.4 ± 0.3^{a}	3.6 ± 0.9^{a}	3.7 ± 0.7^{a}	5.9 ± 0.3^{a}	6.7 ± 0.1^{b}	8.6 ± 0.3^{a}	7.8 ± 0.5^{b}	5.6 ± 0.8^{c}
Inhomogeneous sucrose distribution biscuit	3.1 ± 0.1^{a}	4.2 ± 1.2^{a}	4.1 ± 2.6^{a}	5.7 ± 0.1^{a}	8.1 ± 1.6^{a}	8.5 ± 0.4^{a}	9.2 ± 0.2^{a}	11.0 ± 0.1^{a}	10.3 ± 0.1^{a}
Granulated sugar biscuit	3.2 ± 0.8^{a}	2.7 ± 0.1^{a}	4.0 ± 0.4^{a}	4.5 ± 0.9^{a}	6.9 ± 1.2^{a}	7.2 ± 0.2^{b}	8.5 ± 0.1^{a}	5.5 ± 0.6^{c}	8.0 ± 0.1^{b}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.20. b* value of biscuits with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	30.8 ± 1.8^{a}	29.5 ± 1.6^{b}	29.4 ± 0.9^{b}	30.5 ± 1.1^{b}	31.1 ± 1.4^{b}	31.2 ± 0.9^{b}	30.2 ± 1.2^{b}	31.9 ± 0.8^{b}	29.1 ± 0.6^{b}
Inhomogeneous sucrose distribution biscuit	35.8 ± 3.9^{a}	39.0 ± 0.3^{a}	39.8 ± 5.8^{a}	43.9 ± 1.5^{a}	47.3 ± 1.7^{a}	42.9 ± 2.4^{a}	43.5 ± 1.5^{a}	46.6 ± 0.4^{a}	45.1 ± 1.8^{a}
Granulated sugar biscuit	34.6 ± 1.1^{a}	28.8 ± 0.1^{b}	34.5 ± 1.3^{ab}	32.8 ± 2.6^{b}	36.0 ± 0.1^{b}	35.7 ± 1.9^{ab}	34.1 ± 0.8^{b}	32.6 ± 0.7^{b}	33.6 ± 0.1^{b}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.21. Browning ratio of biscuits with different forms of sucrose*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{b}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}
Inhomogeneous sucrose distribution biscuit	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.5 ± 0.2^{a}
Granulated sugar biscuit	0.2 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.1^{b}	0.3 ± 0.1^{b}	0.4 ± 0.1^{a}	0.2 ± 0.1^{b}	0.3 ± 0.1^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

4.4.3. Effect of the addition of flavour compounds

The hardness values of aroma-added biscuit doughs and biscuits are given in **Table 4.22 and 4.23**. Interestingly, the addition of flavouring agents at sugar concentrations above 12% generally reduced the hardness of the dough although it does not affect the hardness of the biscuits ($p>0.05$). However, ethylvanillin-added biscuits above 37%, furaneol-added biscuits above 34%, and phenylacetaldehyde-added biscuits above 28% sucrose content were not able to be broken by the texture analyzer with the given parameters.

The weight of the aroma-added biscuits and the control were given in **Table 4.24** and the spread ratios are in **Table 4.25**. No significant difference ($p>0.05$) was found in the weight of biscuits above 8% sucrose content and spread ratio except for the biscuits containing 28% sucrose.

Table 4.22. Hardness of aroma-added biscuit doughs*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	12.8 ± 0.5^{b}	9.6 ± 0.7^{b}	8.6 ± 0.4^{ab}	9.0 ± 0.6^{a}	8.0 ± 1.1^{a}	8.3 ± 0.7^{a}	9.2 ± 0.1^{a}	11.9 ± 0.8^{a}	11.8 ± 0.8^{a}
Ethylvanillin-added biscuit	14.2 ± 1.1^{ab}	8.8 ± 0.8^{b}	5.8 ± 0.4^{b}	2.9 ± 0.4^{c}	3.4 ± 0.5^{b}	4.0 ± 0.2^{c}	5.3 ± 0.5^{b}	6.8 ± 0.7^{b}	10.2 ± 3.2^{a}
Furaneol-added biscuit	13.9 ± 1.5^{ab}	7.3 ± 0.1^{b}	6.8 ± 0.6^{b}	3.4 ± 0.4^{c}	3.3 ± 0.3^{b}	4.7 ± 0.1^{b}	5.7 ± 0.9^{b}	6.7 ± 0.4^{b}	7.7 ± 1.9^{a}
Phenylacetaldehyde-added biscuit	16.3 ± 0.3^{a}	14.4 ± 2.1^{a}	9.1 ± 1.6^{a}	5.8 ± 0.6^{b}	4.6 ± 0.1^{b}	5.9 ± 0.1^{b}	7.6 ± 0.4^{a}	9.6 ± 0.5^{a}	9.5 ± 2.3^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.23. Hardness of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	18.1 ± 4.7^{a}	18.6 ± 4.2^{a}	20.6 ± 1.4^{a}	45.4 ± 3.9^{a}	39.8 ± 3.1^{a}	43.3 ± 4.9^{a}	37.9 ± 2.3	49.5 ± 0.5	33.1 ± 5.6
Ethylvanillin-added biscuit	13.4 ± 1.4^{a}	13.5 ± 1.5^{a}	15.9 ± 1.0^{b}	23.6 ± 2.2^{b}	32.2 ± 6.1^{a}	42.3 ± 1.9^{a}	43.1 ± 1.7	-	-
Furaneol-added biscuit	15.5 ± 2.9^{a}	16.5 ± 0.6^{a}	24.5 ± 0.6^{a}	25.5 ± 0.6^{b}	35.2 ± 3.2^{a}	39.2 ± 7.3^{a}	-	-	-
Phenylacetaldehyde-added biscuit	13.4 ± 1.8^{a}	19.5 ± 3.0^{a}	25.1 ± 1.8^{a}	40.6 ± 2.6^{a}	38.2 ± 2.2^{a}	-	-	-	-

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.24. Weight of aroma-added biscuits*

Biscuit	Sugar content (%)								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	8.9 ± 0.1^{b}	9.2 ± 0.1^{b}	9.2 ± 0.2^{a}	9.4 ± 0.3^{ab}	9.5 ± 0.2^{a}	10.0 ±0.4^{a}	9.7 ± 0.1^{a}	10 ± 0.2^{a}	10.1 ± 0.3^{a}
Ethylvanillin-added biscuit	9.8 ± 0.4^{a}	9.6 ± 0.1^{b}	9.2 ± 0.1^{a}	9.3 ± 0.1^{ab}	9.6 ± 0.2^{a}	9.6 ± 0.4^{a}	10.6 ± 0.2^{a}	10.2 ± 0.4^{a}	10.1 ± 1.0^{a}
Furaneol-added biscuit	9.9 ± 0.2^{a}	9.8 ± 0.1^{b}	9.8 ± 0.3^{a}	9.2 ± 0.1^{b}	9.2 ± 0.1^{a}	9.4 ± 0.1^{a}	9.6 ± 0.1^{a}	10.1 ± 0.1^{a}	10.1 ± 0.4^{a}
Phenylacetaldehyde-added biscuit	9.8 ± 0.1^{a}	9.8 ± 0.1^{a}	10.1 ± 0.6^{a}	9.8 ± 0.2^{a}	9.6 ± 0.1^{a}	10.0± 0.2^{a}	10.7 ± 0.7^{a}	10.4 ± 0.5^{a}	10.3 ± 0.3^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

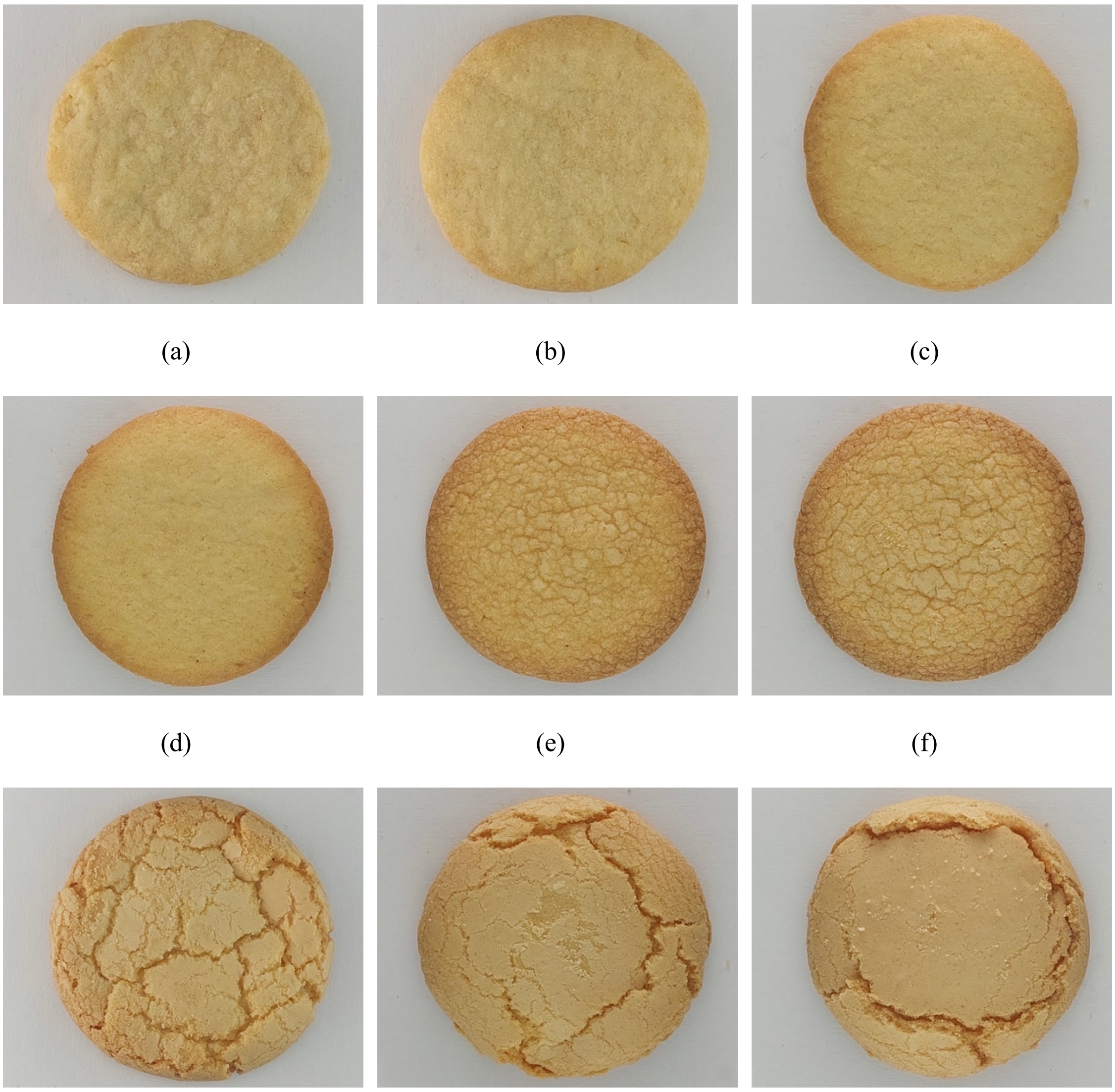
Table 4.25. Spread ratio of aroma-added biscuits*

Biscuit	Sugar content (%)								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	4.6 ± 0.3^{a}	4.8 ± 0.1^{a}	4.8 ± 0.2^{a}	6.3 ± 0.3^{a}	5.7 ± 0.2^{a}	6.8 ± 0.2^{a}	7.0 ± 0.1^{a}	5.4 ± 1.0^{a}	4.0 ± 0.3^{a}
Ethylvanillin-added biscuit	4.5 ± 0.1^{a}	4.8 ± 0.1^{a}	5.0 ± 0.2^{a}	5.7 ± 0.2^{a}	6.0 ± 0.7^{a}	6.3 ± 0.2^{b}	6.0 ± 0.2^{a}	4.2 ± 0.1^{a}	3.4 ± 0.2^{a}
Furaneol-added biscuit	4.7 ± 0.4^{a}	5.1 ± 0.1^{a}	4.9 ± 0.1^{a}	5.8 ± 0.1^{a}	5.9 ± 0.1^{a}	6.4 ± 0.1^{ab}	6.7 ± 0.5^{a}	3.7 ± 0.1^{a}	3.5 ± 0.1^{a}
Phenylacetaldehyde-added biscuit	5.1 ± 0.3^{a}	4.8 ± 0.1^{a}	4.9 ± 0.4^{a}	5.7 ± 0.3^{a}	5.8 ± 0.1^{a}	5.8 ± 0.4^{b}	7.0 ± 0.3^{a}	3.3 ± 0.2^{a}	3.4 ± 0.1^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

L*, a*, and b* values and the browning ratios of aroma-added biscuits were shown in **Table 4.26-29**, which were obtained using the digital images examples of which are given in **Figure 4.14-16**.

Changes in L* values with aroma addition were shown in **Table 4.26**. L values increased at the sucrose concentrations of 8, 23, and 34%. a* values slightly increased at the sucrose concentrations of 17-28% compared to control (**Table 4.27**). b* values were decreased with aroma addition at each sucrose concentration (**Table 4.28**). The browning ratios of aroma-added biscuits were shown in **Table 4.29**. The browning ratios of aroma-added biscuits decreased at 8, 12, 23, and 28% sucrose concentrations.

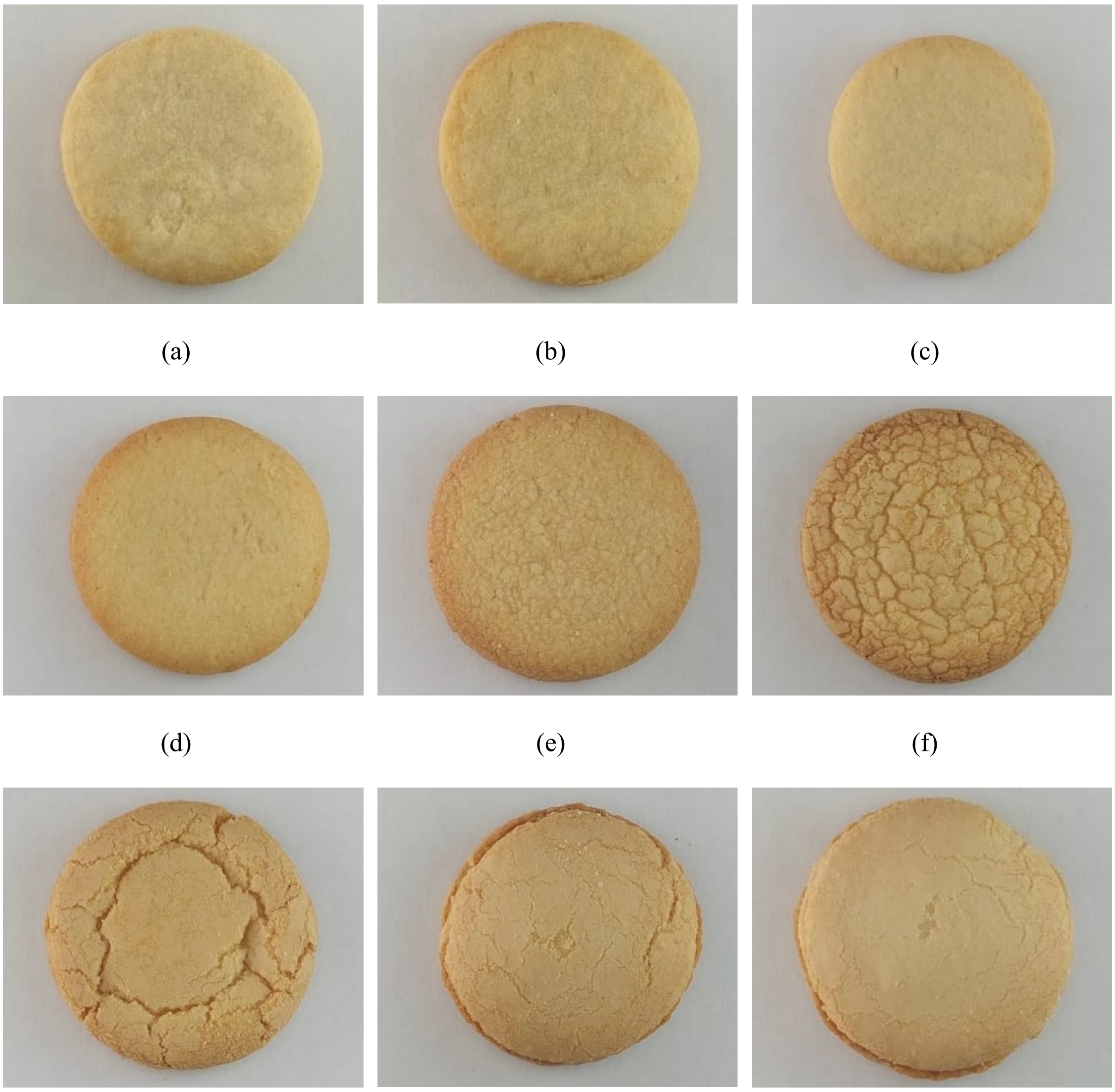


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.14. Example of ethylvanillin-added biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.

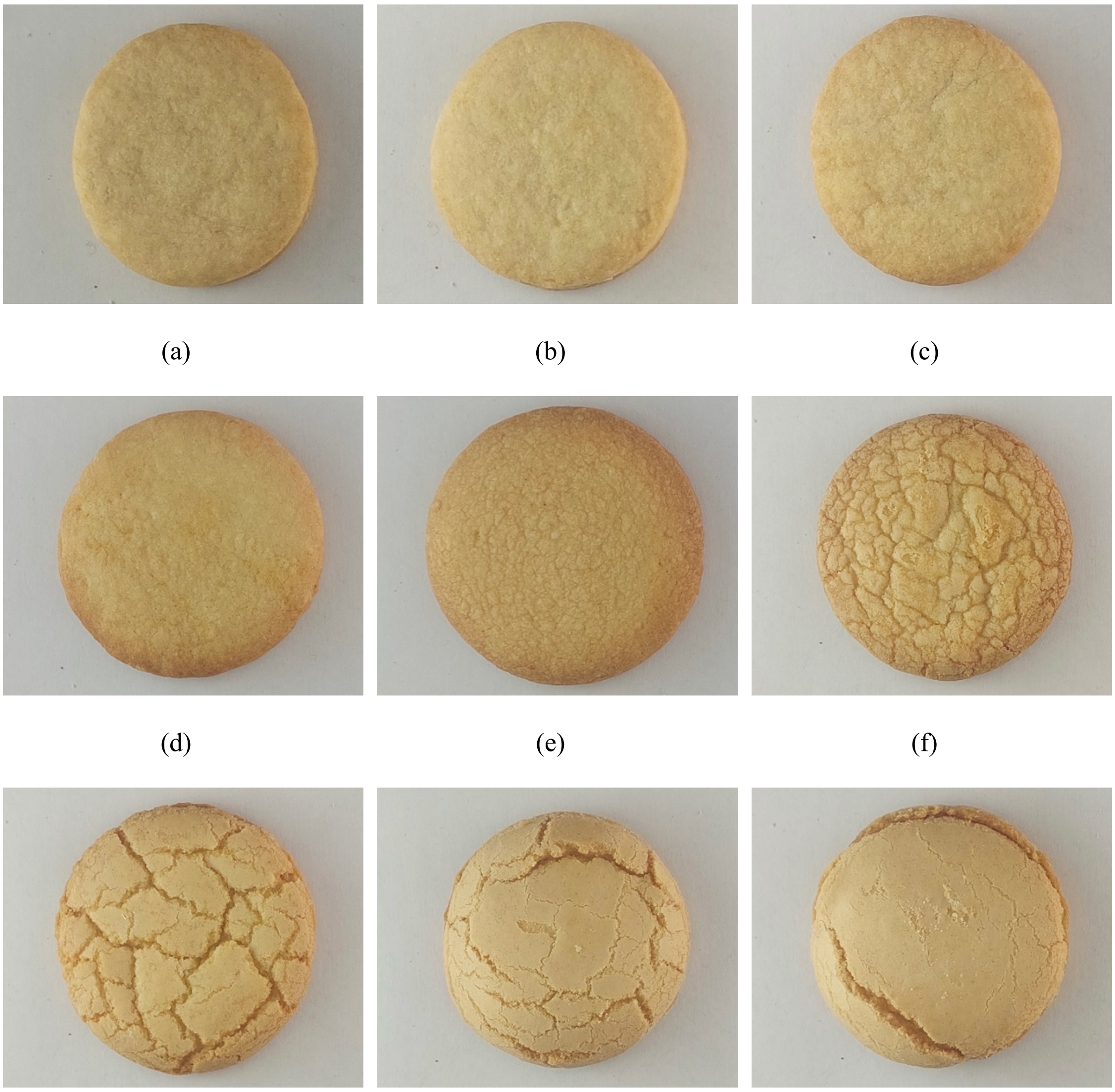


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.15. Example of furaneol-added biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 4.16. Example of phenylacetaldehyde-added biscuits containing (a) 6%, (b) 8%, (c) 12%, (d) 17%, (e) 23%, (f) 28%, (g) 34%, (h) 37%, (i) 39% sucrose

Table 4.26. L* value of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	62.5 ± 0.2^{a}	61.7 ± 0.3^{b}	61.4 ± 1.6^{a}	61.4 ± 0.2^{a}	58.6 ± 0.4^{b}	58.4 ± 0.8^{b}	55.5 ± 2.1^{c}	57.6 ± 1.2^{a}	59.7 ± 2.1^{a}
Ethylvanillin-added biscuit	67.6 ± 0.5^{a}	67.6 ± 1.4^{a}	65.2 ± 2.6^{a}	63.5 ± 2.5^{a}	62.2 ± 0.1^{a}	60.3 ± 2.3^{ab}	61.9 ± 3.6^{ab}	62.1 ± 1.0^{b}	64.3 ± 1.4^{a}
Furaneol-added biscuit	66.5 ± 4.4^{a}	68.2 ± 0.1^{a}	68.5 ± 0.9^{a}	66.8 ± 0.5^{a}	61.8 ± 2.4^{a}	60.9 ± 1.4^{ab}	64.3 ± 0.1^{ab}	64.2 ± 1.6^{ab}	64.1 ± 2.9^{a}
Phenylacetaldehyde-added biscuit	66.0 ± 4.6^{a}	70.6 ± 0.8^{a}	68.6 ± 2.2^{a}	66.1 ± 2.9^{a}	64.2 ± 3.4^{a}	64.5 ± 0.9^{a}	66.3 ± 1.8^{a}	68.1 ± 0.4^{a}	67.6 ± 0.2^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.27. a* value of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	2.7 ± 0.4^{a}	3.4 ± 0.3^{a}	3.6 ± 0.9^{a}	3.7 ± 0.7^{b}	5.9 ± 0.3^{b}	6.7 ± 0.1^{c}	8.6 ± 0.3^{a}	7.8 ± 0.5^{a}	5.6 ± 0.8^{b}
Ethylvanillin-added biscuit	3.6 ± 0.4^{a}	3.4 ± 0.2^{a}	4.6 ± 0.8^{a}	6.2 ± 0.6^{a}	9.6 ± 0.3^{a}	9.9 ± 0.7^{a}	9.8 ± 2.4^{a}	8.3 ± 0.3^{a}	8.2 ± 0.8^{a}
Furaneol-added biscuit	2.4 ± 0.8^{a}	2.9 ± 0.2^{a}	2.9 ± 0.9^{a}	5.0 ± 0.1^{ab}	8.0 ± 0.8^{ab}	9.2 ± 0.1^{ab}	8.3 ± 0.1^{a}	7.0 ± 0.3^{ab}	5.4 ± 0.5^{b}
Phenylacetaldehyde-added biscuit	3.2 ± 0.7^{a}	2.5 ± 0.3^{a}	3.5 ± 0.9^{a}	5.8 ± 0.6^{ab}	7.8 ± 0.6^{ab}	8.3 ± 0.1^{b}	7.3 ± 1.1^{a}	5.5 ± 0.5^{b}	5.9 ± 0.1^{ab}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.28. b* value of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	30.8 ± 1.8^{b}	29.5 ±1.6^{b}	29.4 ± 0.9^{b}	30.5 ± 1.1^{c}	31.1 ± 1.4^{b}	31.2 ±0.9^{b}	30.2 ±1.2^{b}	31.9 ± 0.8^{b}	29.1 ± 0.6^{c}
Ethylvanillin-added biscuit	36.7 ± 1.5^{a}	38.4 ± 0.9^{a}	40.0 ± 0.8^{a}	42.8 ± 0.1^{a}	42.5 ± 0.1^{a}	40.6 ± 0.7^{a}	39.2 ± 2.3^{a}	38.5 ± 0.1^{a}	36.6 ± 1.2^{a}
Furaneol-added biscuit	34.8 ± 0.8^{ab}	37.8 ± 0.6^{a}	36.3 ± 3.7^{ab}	38.6 ±0.6^{b}	38.3 ± 3.4^{ab}	37.7 ± 0.4^{a}	37.7 ± 0.7^{a}	35.1 ± 1.4^{ab}	31.6 ± 0.2^{bc}
Phenylacetaldehyde-added biscuit	34.6 ± 0.8^{ab}	35.6 ± 1.4^{a}	35.5 ± 1.1^{ab}	36.9 ±0.7^{b}	36.3 ± 0.5^{ab}	37.2 ± 2.3^{a}	37.8 ± 1.0^{a}	32.7 ± 1.0^{b}	34.9 ± 1.1^{ab}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

Table 4.29. Browning ratio of aroma-added biscuits*

Biscuit	**Sugar content (%)**								
	6	**8**	**12**	**17**	**23**	**28**	**34**	**37**	**39**
Control biscuit	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}
Ethylvanillin-added biscuit	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{b}	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{ab}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}
Furaneol-added biscuit	0.2 ± 0.1^{a}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}	0.1 ± 0.1^{a}	0.1 ± 0.1^{b}	0.2 ± 0.1^{b}	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	0.1 ± 0.1^{a}
Phenylacetaldehyde-added biscuit	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.2 ± 0.1^{b}	0.1 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.2 ± 0.1^{b}	0.3 ± 0.1^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}

* In a column, scores with the same lowercase letters are not statistically different according to Tukey's test at p=0.05.

5. CONCLUSION

Biscuit is one of the most preferred processed foods in daily routine because of its being ready-to-eat, easy accessibility, and long shelf life. Due to health concerns, sucrose, which is one of the main components of the biscuit recipe, is desired to be reduced in the daily diet and various strategies are being developed for sugar reduction. Sugar reduction in biscuits is a major challenge because of the critical effects of sucrose on the sweetness, flavour, and physical properties of biscuits. Furthermore, not using a specific model in determining the perception of sugar density in biscuits makes it challenging to compare the data obtained from studies on this subject with each other. Moreover, in some cases, the models for determining sweetness perception are not enough to define sweetness perception's lower and upper limits.

In this study, the modified Weibull model was used for the first time to predict the sweetness perception of biscuits. Biscuits with different formulations were baked and the perceived sweetness in these biscuits against varying sucrose concentrations was found to fit the modified Weibull model very well. Once the sigmoidal curve is obtained for a biscuit formulation using the sensory analysis results and the modified Weibull model, the perception of sweetness corresponding to any sugar concentration for this biscuit could be predicted without resorting to sensory analysis. This approach may help the industry in reformulation studies in terms of sugar reduction once the sugar concentration versus sweetness perception catalogue was created for each biscuit formulation.

Sensory analysis and modelling results both showed that the perceived sweetness in biscuits did not change above and below certain sugar concentrations. Upper and lower sugar concentration limits were determined for biscuits, where the perception of sweetness did not change. It was concluded that the differences in the physical properties of the biscuits did not affect the perceived sweetness. Adding wholewheat flour, protein, and sweetness-related flavourings to biscuits have been found to increase perceived sweetness. It was thought that the increased sweetness when wholewheat flour and protein were added was due to the increase in the amount of volatile compounds formed.

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ANNEX

ANNEX 1 – Surveys

Gönüllü Katılım Formu

Katılacağınız duyusal değerlendirme paneli Prof. Dr. Vural Gökmen danışmanlığında yürütülen 'Bisküvilerde şeker azaltmanın duyusal analiz temelli incelenmesi' başlıklı yüksek lisans tezi (yüksek lisans öğrencisi Naz Erdem) ve ayrıca yürütücülüğünü Prof. Dr. Vural Gökmen'in yaptığı TUBITAK 120N061 nolu projenin bir kısmı kapsamında gerçekleştirilmektedir.

Bu çalışma sırasında sizden bisküviler tatmanızı ve bunların tatlılık seviyeleri ile ilgili size verilecek ölçek üzerinde işaretleme yapmanız beklenecektir. Elde edilen veriler farklı bisküvilerin tatlılık algılarının matematiksel modellenmesi amacıyla kullanılacaktır.

Çalışmaya katılım gönüllülük esasına dayanmaktadır.

Çalışma devam ederken istediğiniz anda ayrılabilirsiniz ve bu size hiçbir sorumluluk getirmez.

Çalışma ile ilgili sorularınız için çalışma esnasında ve sonrasında araştırmacılarla telefon veya e-posta yolu ile iletişime geçerek bilgi alma hakkınız bulunmaktadır.

Kimlik bilgileriniz kimse ile paylaşılmayacak olup sizden yalnızca birbirini takip eden duyusal değerlendirmelerde aynı panelist numarasını kullanmanız istenecektir. Bu panelist numaraları istatiksel değerlendirme için gerekli olup hiçbir şekilde buradaki kişisel bilgilerinizle eşleşmeyecektir. Araştırma sonuçları TÜBİTAK raporu, başlığı yukarıda belirtilen tez ve ilişkili yayınlarda yer alacak ve hiçbir şekilde kişisel bilgilerinizi içermeyecektir.

Lütfen aşağıdaki soruları dikkatlice cevaplayınız ve sorunuz varsa araştırmacı ile iletişime geçiniz.

Herhangi bir gıda alerjiniz var mı? ☐Evet ☐Hayır
Varsa tümünü belirtiniz:

Herhangi bir gıda intoleransınız var mı? ☐Evet ☐Hayır
Varsa tümünü belirtiniz:

Her ne gerekçe ile olursa olsun yemeği tercih etmediğiniz/reddettiğiniz bir gıda var mı? ☐ Evet ☐Hayır
Varsa tümünü belirtiniz:

Panelist No:
Tarih:

Bisküvilerde Tatlılık Algısı Paneli

Sayın Panelist,

Aşağıda size sunulan örnekleri bu sayfadaki numara sırasına göre tatmanızı ve size en doğru gelen TATLILIK (ŞEKER TADI) seviyesini çizgi ölçeğini kesecek şekilde işaretlemenizi rica ederiz.

Çizgi ölçeğini günlük tatlılık deneyiminize uygun şekilde kullanabilirsiniz. Çizginin sol ucu size göre hiç tatlı olmayan duyusal seviyeyi (TATLI DEĞİL), sağ ucu ise hayatınızda deneyimlediğiniz en tatlı seviyeyi (AŞIRI TATLI) göstermektedir.

Örnek aralarında eşit süre beklemek için lütfen telefonunuzdaki kronometreyi kullanınız.

Örnekleri sadece bir kez tadınız ve size en doğru gelen yere işaret koyunuz!

Örnek: **381** Tatlı değil ├────────────────────┤ Aşırı tatlı

Yeterince su ile ağzınızı çalkayın, kronometreyi başlatın ve 30 saniye bekledikten sonra diğer örneğe geçin.

Örnek: **105** Tatlı değil ├────────────────────┤ Aşırı tatlı

Yeterince su ile ağzınızı çalkayın, kronometreyi başlatın ve 30 saniye bekledikten sonra diğer örneğe geçin.

Örnek: **423** Tatlı değil ├────────────────────┤ Aşırı tatlı

Yeterince su ile ağzınızı çalkayın, kronometreyi başlatın ve 30 saniye bekledikten sonra diğer örneğe geçin.

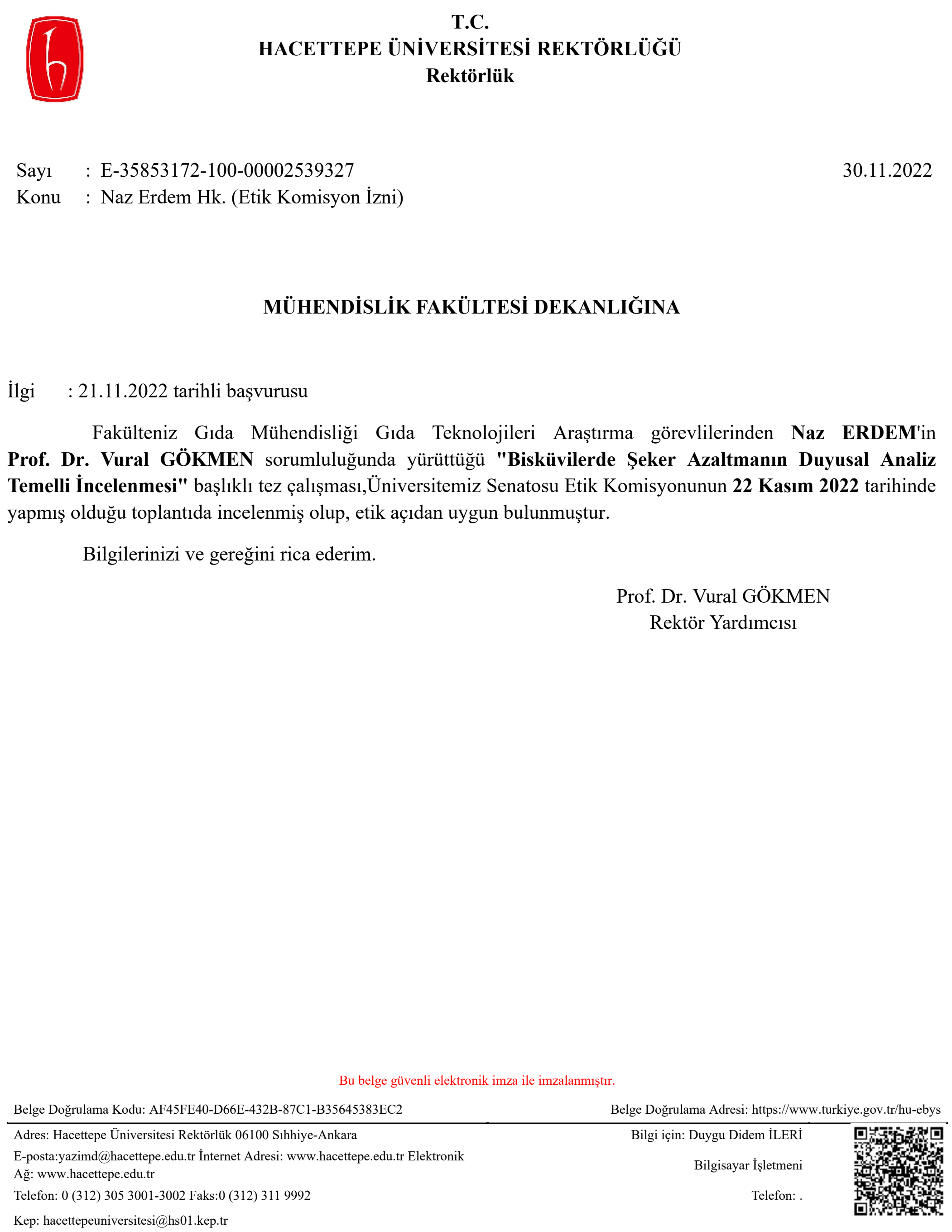
Örnek: **246** Tatlı değil ├────────────────────┤ Aşırı tatlı

Yeterince su ile ağzınızı çalkayın, kronometreyi başlatın ve 30 saniye bekledikten sonra diğer örneğe geçin.

Örnek: **589** Tatlı değil ├────────────────────┤ Aşırı tatlı

Bu paneldeki örnekleri tamamladınız. Teşekkürler.

ANNEX 2 – Ethics Committee Permission Certificate



T.C.
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MÜHENDİSLİK FAKÜLTESİ DEKANLIĞINA

İlgi : 21.11.2022 tarihli başvurusu

Fakülteniz Gıda Mühendisliği Gıda Teknolojileri Araştırma görevlilerinden **Naz ERDEM**'in **Prof. Dr. Vural GÖKMEN** sorumluluğunda yürüttüğü **"Bisküvilerde Şeker Azaltmanın Duyusal Analiz Temelli İncelenmesi"** başlıklı tez çalışması,Üniversitemiz Senatosu Etik Komisyonunun **22 Kasım 2022** tarihinde yapmış olduğu toplantıda incelenmiş olup, etik açıdan uygun bulunmuştur.

Bilgilerinizi ve gereğini rica ederim.

Prof. Dr. Vural GÖKMEN
Rektör Yardımcısı

Bu belge güvenli elektronik imza ile imzalanmıştır.

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ANNEX 3 - Publications

N. Erdem, N. Göncüoğlu Taş, T. Kocadağlı, V. Gökmen, Modelling of Perceived Sweetness in Biscuits Based on Sensory Analysis as a New Tool to Evaluate Reformulation Performance in Sugar Reduction Studies, *Food Chem* (2022). The manuscript is under review.

ANNEX 4 – Oral and Poster Presentations

Erdem, N., Göncüoğlu Taş, N., Kocadağlı, T., Gökmen, V., Modelling of perceived sweetness in biscuits based on sensory analysis as a new tool to evaluate reformulation performance in sugar reduction studies, 9th Nursten Postgraduate Flavour Symposium, 20-21 June, Reading, England, 2022.

Erdem, N., Göncüoğlu Taş, N., Kocadağlı, T., Gökmen, V., Modelling of perceived sweetness in biscuits to evaluate reformulation performance in sugar reduction studies, Effost 2022 International Conference, 7-9 November, Dublin, Ireland, 2022.