PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

SCHOOL OF ENGINEERING

# ROTARY-MOULDED BISCUITS: UNDERSTANDING THE EFFECT OF SUGAR CONCENTRATION AND WHEAT BRAN ENRICHMENT ON KEY QUALITY ATTRIBUTES TO DEVELOP HEALTHIER OPTIONS

**MARÍA TERESA MOLINA MAYDL**

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Advisors:

**PEDRO BOUCHON AGUIRRE**

**BALTASAR VALLÈS-PÀMIES**

Santiago de Chile, January 2021

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
SCHOOL OF ENGINEERING

# ROTARY-MOULDED BISCUITS: UNDERSTANDING THE EFFECT OF SUGAR CONCENTRATION AND WHEAT BRAN ENRICHMENT ON KEY QUALITY ATTRIBUTES TO DEVELOP HEALTHIER OPTIONS

**MARÍA TERESA MOLINA MAYDL**

Members of the Committee:

**PEDRO BOUCHON AGUIRRE**

**BALTASAR VALLÈS-PÀMIES**

**WENDY FRANCO MELAZZINI**

**FRANCO PEDRESCHI PLASENCIA**

**VILBETT BRIONES LABARCA**

**LISA LAMOTHE**

**YADRAN ETEROVIC SOLANO**

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree Doctor in Engineering Sciences

Santiago de Chile, January 2021

To my husband, Rafael,
my parents, M. Teresa, Mario,
and my siblings, M. Isabel, Mario.

**ACKNOWLEDGEMENTS**

First of all, I would like to thank to my advisors Doctor Pedro Bouchon, Doctor Baltasar Vallès-Pàmies, and Doctor Sandra Vaz. To professor Pedro for his constant guidance, encouraging meetings, inspiring questions, and his unconditional support during the most challenging moments. To Baltasar for believing in the *particular biscuit* that was key to initiate a patent process. To Sandra for opening me the doors of Nestlé Development Center to develop my PhD research.

To the members of the committee Doctor Franco Pedreschi, Doctor Wendy Franco, Doctor Vilbett Briones, Doctor Lisa Lamothe, and Doctor Yadran Eterovic, thank for your comments, questions and suggestions which helped to me to improve my research.

I am also very grateful to Lisa Lamothe and Deniz Gunes for giving me valuable advice during my internship at the Nestlé Research. In addition, I owe my thanks to Christine, Delphine, Sylvie, Andrea and Yu-Jie for their willingness to help me at every moment, for making me feel at home in Switzerland, and for their special farewell and gift before I come back to Chile.

To all the people that was part of the NDC team during my time there, and especially to Patricio, Rodrigo, Tamara, Javiera, Betza, Caio, Javier and Diana, thank for your constant support, energy and motivation, which were crucial at all times.

I would also like to express my gratitude to Mane Valdebenito and Roberto Caiseo for their invaluable help on handling the release of funds during the development of my thesis.

To my friends, Chris F., Eli C., Denisse S., Denisse F., Pauli T., Ingrid C., Sole M., Caro S., Jaime V., Lore V., Trini S., and Javi R., I should like to thank for their friendship, advice, and company, which were crucial for bringing this writing to an end.

Finally, I would like to express my biggest gratitude to Rafael, my parents M. Teresa and Mario, and my siblings, Isa and Mario, for their unconditional faith, motivation, patience and encouragement to finish this unforgettable process.

*Financial support:*

I would like to acknowledge the financial support of CONICYT through the Advanced National Human Capital Formation Program - Doctoral Fellowship 2016 (Folio n° 21161243), Vice-rectory of Research UC (VRI), the School of Engineering of Pontificia Universidad Católica de Chile, and the NESTLÉ-UC University Research Agreement.

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

**ROTARY-MOULDED BISCUITS: UNDERSTANDING THE EFFECT OF SUGAR CONCENTRATION AND WHEAT BRAN ENRICHMENT ON KEY QUALITY ATTRIBUTES TO DEVELOP HEALTHIER OPTIONS**

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences by

**MARÍA TERESA MOLINA MAYDL**

## ABSTRACT

Consumers' growing concerns about nutrition and excessive caloric intake has moved their choices to favor healthier biscuits development, imposing a new challenge on biscuit manufacturers. Reformulating ingredients to produce fiber-rich or sugar-and-fat-reduced biscuits usually compromises biscuit texture and flavor, and consumers expect to have the same sensory experience as one they get when consuming indulgent biscuits. Therefore, it is relevant for the industry to further study the possibilities of such reformulations.

The main objective of this research is to understand the impact of the creaming stability, the sucrose reduction and the wheat bran enrichment on the structure development during processing of rotary-moulded biscuits, in order to modulate (micro) and (macro) structural quality attributes such as the aeration level, the sweetness perception and the texture hardness. For this purpose, a microstructural approach was conceived to ascertain the link between sensory attributes and biscuit properties at micro and macro-structural levels.

Rotary-moulded dough was formulated using soft wheat flour (44-74%, d.b.) or whole flour (~65%, d.b.), sucrose (10-40%, d.b.), fat (~12%, d.b.), water (7-16%, d.b.), leavening agents (~2.5%, d.b.), lecithin (~0.2%, d.b.), and salt (~0.1%, d.b.). The creaming was prepared using a low-shear (~166 rpm) or high-shear mixer (~9000 rpm) in order to study the effect of its stability on quality attributes of rotary-moulded biscuits. Also, two sucrose

particle sizes ($D_{90} = 978\ or\ 98\ \mu m$) and four [sucrose:flour] ratios (0.9, 0.5, 0.3, or 0.1) were proposed to analyze the effect of sucrose reduction on dough expansion during baking and on the resultant structure of biscuits. In addition, soft wheat flour was replaced at 25, 50, 75, or 100% by whole flour with different bran particle sizes ($D_{Sauter} = 790\ or\ 307\ \mu m$) to evaluate the influence of wheat bran on dough rheology and thermal behavior during processing of rotary-moulded biscuits. The firmness of dough was analyzed using a texture analyzer, and frequency sweep tests were performed for dough rheology analysis. The dough expansion during baking was monitored by time-lapse photography, and the degree of starch gelatinization was determined using differential scanning calorimetry. The microstructure of biscuits was observed and quantified using X-ray micro-computed tomography and scanning electron microscopy. Sensory attributes and sweetness perception were characterized with a trained sensory panel.

The results showed that an aqueous-phase migration occurred when the creaming was blended in a low-shear mixer, and it was inhibited with a high-shear mixer, which provided a stable creaming. Notwithstanding this variation, no differences were observed in hardness, aeration, sweetness, color and noise intensity of rotary-moulded biscuits. Related to sucrose reduction in rotary-moulded biscuits, the results not only revealed that a partial gelatinization of starch granules occurred in all formulations, but also that the degree of gelatinized starch significantly increased from 6% to 40% as the sucrose content decreased from 40% to 10% (d.b.). This behavior was explained by the antiplasticizing effect of the sucrose-water cosolvent, suggesting that it retarded the sequential thermal events of gelatinization. On the other hand, the sucrose particle size was a key variable to modulate the vertical expansion of rotary-moulded doughs. Biscuits that were formulated with powdered sucrose and more than 30% of added sucrose reached an expanded and non-collapsible structure. This aerated structure has not been seen before in this biscuit category, so that a patent was filed from this knowledge. It was suggested that the sucrose dissolution was relevant to prolong the vertical expansion of dough because, as the concentration of the sugar solution increases, the thermal transitions of starch and proteins are retarded, which are mainly responsible for controlling the dough expansion. Regarding the fiber-enriched biscuits, wheat bran had the greatest impact on dough firmness, while arabinoxylans had the greatest impact on elastic response of the dough. Also, the degree of gelatinized starch increased from 24 to 36% in biscuits enriched with arabinoxylans or whole flour and large bran. A microstructural perspective was used to explain this result, and it was proposed that the micropores of the wheat bran insoluble part may retain water inside their capillaries, which then the water can be released in a controlled manner during baking, allowing starch granules to partially undergo a thermal transition as cooking progresses.

From this thesis, it suggests that the stability of the creaming phase does not seem to be a relevant factor to determine the quality attributes of rotary-moulded biscuits, when a representative formulation for this product category was used. Furthermore, this research showed that the sugar reduction promoted the starch gelatinization. Soluble fibers may be an interesting alternative to be used as bulking agents due to their lower water holding capacity compared to flour. Finally, the processing of rotary-moulded doughs was not affected by whole flour incorporation. However, the gelatinization of starch granules increased when a porous structure of bran was employed, so that micronized wheat bran could be an alternative to control this phenomenon and, consequently, to reduce the glycemic impact of consuming bran-enriched biscuits.

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

# GALLETA MOLDEADA: COMPRENSIÓN DEL EFECTO DE LA CONCENTRACIÓN DE AZÚCAR Y EL ENRICHECIMIENTO DE SALVADO DE TRIGO EN ATRIBUTOS DE CALIDAD CLAVE PARA DESARROLLAR OPCIONES MÁS SALUDABLES

Tesis enviada a la Dirección de Postgrado en cumplimiento parcial de los requisitos para el grado de Doctor en Ciencias de la Ingeniería.

**MARÍA TERESA MOLINA MAYDL**

## RESUMEN

La creciente preocupación de los consumidores sobre la nutrición y la excesiva ingesta calórica ha cambiado sus opciones hacia galletas más saludables, planteando así un nuevo desafío para la industria de galletas. La reformulación de ingredientes para obtener galletas ricas en fibra o reducidas en azúcar y grasa generalmente compromete su textura y sabor, y los consumidores esperan tener la misma experiencia sensorial que tendrían con las galletas indulgentes. Por lo tanto, es relevante para la industria continuar estudiando las posibilidades de tales reformulaciones.

El objetivo principal de esta investigación es comprender el impacto de la estabilidad del *creaming*, la reducción de sacarosa y el enriquecimiento del salvado de trigo en el desarrollo de la estructura durante el procesamiento de galleta moldeada, con el propósito de modular atributos de calidad (micro) y (macro) estructurales, como el nivel de aireación, la percepción de dulzor y la dureza. Para ello, se concibió un enfoque microestructural con el fin de determinar el vínculo entre los atributos sensoriales y las propiedades de las galletas a nivel micro y macroestructural.

La masa de galleta moldeada se formuló con harina de trigo débil (44-74%, b.s.) o harina integral (~65%, b.s.), sacarosa (10-40%, b.s.), grasa (~12%, b.s.), agua (7-16%, b.s.), agentes

leudantes (~2.5%, b.s.), lecitina (~0.2%, b.s.) y sal (~0.1%, b.s.). El *creaming* se preparó en un mezclador de bajo (~166 rpm) o alto cizallamiento (~9000 rpm) para estudiar el efecto de su estabilidad en los atributos de calidad de galleta moldeada. Además, se utilizó sacarosa con dos tamaños de partícula ($D_{90} = 978\ o\ 98\ \mu m$) y cuatro relaciones [sacarosa: harina] (0.9, 0.5, 0.3, 0.1) para analizar el efecto de la reducción de sacarosa en la expansión de la masa durante el horneado y en la estructura de las galletas. Además, la harina de trigo débil se reemplazó por harina de trigo integral al 25, 50, 75 ó 100%, utilizando diferentes tamaños de partícula de salvado ($D_{Sauter} = 790\ o\ 307\ \mu m$) para evaluar la influencia del salvado de trigo en la reología de la masa y en el comportamiento térmico durante el procesamiento de galleta moldeada. La firmeza de masa se analizó mediante un texturómetro, y se realizaron pruebas de barrido de frecuencia para el análisis reológico. La expansión de la masa durante el horneado se monitoreó a través de fotografía a intervalos, y el grado de gelatinización del almidón se determinó usando calorimetría diferencial de barrido. La microestructura de las galletas se observó y cuantificó mediante microtomografía computarizada de rayos X y microscopía electrónica de barrido. Los atributos sensoriales y la percepción de dulzor se caracterizaron con un panel sensorial entrenado.

Los resultados mostraron que se produjo una migración de fase acuosa cuando el *creaming* se elaboró en un mezclador de bajo cizallamiento, y se inhibió al utilizar un mezclador de alto cizallamiento, proporcionando un *creaming* estable. A pesar de esta variación, no se observaron diferencias en la dureza, aireación, dulzura, color e intensidad del ruido de galletas moldeadas. En relación con la reducción de sacarosa en galletas moldeadas, los resultados no solo mostraron que se produjo una gelatinización parcial de los gránulos de almidón en todas las formulaciones, sino también que el grado de almidón gelatinizado aumentó significativamente de 6% a 40% a medida que el contenido de sacarosa disminuyó de 40% a 10. % (b.s.). Este comportamiento se explicó por el efecto anti-plastificante del codisolvente sacarosa-agua, sugiriendo que retardó los sucesos térmicos secuenciales de gelatinización. Por otro lado, el tamaño de partícula de la sacarosa fue una variable clave para modular la expansión vertical de las masas de galletas moldeadas. Las galletas que fueron formuladas con sacarosa fina y con más del 30% (b.s.) de sacarosa añadida obtuvieron una estructura expandida y que no colapsó. Esta estructura aireada no se ha visto antes en esta categoría de galletas, por lo que se presentó una patente a partir de este conocimiento. Se sugirió que la disolución de la sacarosa era relevante para prolongar la expansión vertical de la masa porque, a medida que aumenta la concentración de la solución de azúcar, se retardan las transiciones térmicas del almidón y las proteínas, que son las principales responsables de controlar la expansión de la masa. En cuanto a las galletas enriquecidas con fibra, el salvado de trigo tuvo mayor impacto en la firmeza de la masa, mientras que los arabinoxilanos tuvieron mayor impacto en la respuesta elástica de la masa. Además, el grado de almidón gelatinizado aumentó del 24 al 36% en galletas enriquecidas con arabinoxilanos o harina integral y salvado grueso. Se utilizó una perspectiva microestructural para explicar este resultado, y se propuso que los microporos de la parte insoluble del salvado de trigo pueden retener agua dentro de sus capilares, que luego puede ser liberada de manera controlada durante el horneado, permitiendo que los gránulos de almidón experimenten parcialmente una transición térmica a medida que avanza la cocción.

A partir de esta tesis, se sugiere que la estabilidad de *creaming* no parece ser un factor relevante para determinar los atributos de calidad de las galletas moldeadas, cuando se utilizó una formulación representativa para esta categoría de productos. Además, esta investigación mostró que la reducción de azúcar promovió la gelatinización del almidón. Las fibras solubles pueden ser una alternativa interesante para ser utilizadas como agentes de reemplazo debido a su menor capacidad de retención de agua en comparación con la harina. Finalmente, el procesamiento de masas moldeadas no se vio afectado por la incorporación de harina integral. Sin embargo, la gelatinización de los gránulos de almidón aumentó cuando se utilizó una estructura porosa de salvado, por lo que el salvado de trigo micronizado podría ser una alternativa para controlar este fenómeno y, en consecuencia, reducir el impacto glicémico del consumo de galletas enriquecidas con salvado.

**Miembros de la Comisión de Tesis Doctoral:**

**Pedro Bouchon Aguirre**

**Baltasar Vallès-Pàmies**

**Wendy Franco Melazzini**

**Franco Pedreschi Plasencia**

**Vilbett Briones Labarca**

**Lisa Lamothe**

**Yadran Eterovic Solano**

**Santiago, enero, 2021**

## LIST OF CONTENTS

**FIGURE INDEX**

## TABLE INDEX

# ABREVIATION INDEX

| | |
|---|---|
| AX | Arabinoxylans |
| DG | Degree of gelatinization |
| DSC | Differential Scanning Calorimetry |
| FoP | Front-of-package |
| G' | Elastic modulus |
| G" | Loss modulus |
| GS | Granulated sucrose |
| Hor | Low-shear mixing |
| HSh | High-shear mixing |
| kDa | Kilodalton |
| MFL | Model flour simulating whole flour with large bran |
| MFS | Model flour simulating whole flour with small bran |
| PS | Powdered sucrose |
| RF | Refined flour |
| SEM | Scanning electron microscopy |
| SFI | Solid fat index |
| ST | Sugar saturation |
| SRC | Solvent retention capacity |
| $\tan\delta$ | Loss tangent |
| TAX | Total arabinoxylans |
| WEAX | Water-extractable arabinoxylans |
| WFL | Whole flour with large bran fractions |
| WFS | Whole flour with small bran fractions |
| WRC | Water retention capacity |
| WUAX | Water-unextractable arabinoxylans |
| X-ray μCT | X-ray computed microtomography |

# 1. CHAPTER I: Introduction

Sweet biscuits represent the largest category among the global biscuit market. According to the report *Sweet Biscuits Market – Growth, Trends, and Forecasts (2020-2025)* presented by *Research and Market*, this group is projected to grow at a compound annual growth rate of 5.3% during 2020-2025. However, due to the growing nutrition concerns, consumers are not only looking for premium, luxury and indulgent biscuits, but also for healthy biscuits that are fat-and-sugar-reduced, fiber-rich, and gluten free. This new trend has put a new challenge for these companies, which is to produce healthier biscuits while preserving the quality attributes that consumers want to experience: crispiness, crunchiness, low density (lighter bite), and softness.

## 1.1 Classification of biscuits

The classification of biscuits is based on the dough formulation and the method used to shape them, giving rise to the following types of biscuits: deposited, laminated, sugar-snap, rotary-moulded, and wire-cut. **Figure 1.1** presents a ternary diagram illustrating the ratio sugar:fat:flour in biscuits that will be described below, where these three ingredients were adjusted to 100% percentage of dough on a dry basis (d.b.). *Deposited biscuits* are characterized by being prepared from a fluid batter, which is comprised of a high amount of water (or liquid egg in some cases). Also, these biscuits usually have a very low amount of fat (~ 3%, d.b.) but may have a wide range of sugar (from 3 to 60%, d.b.). Examples of them include *Alteza* (wafer by Nestlé McKay) and *Criollitas* (deposited by Nestlé McKay), which have been represented in purple and yellow points in **Figure 1.1**, respectively.

*Laminated biscuits* are made from a viscoelastic and fermented dough, which is formulated with strong flour (11-13% of total protein), no more than 10% of fat (d.b.), and they may or may not contain sugar. The viscoelastic dough is usually subjected to sheeting and relaxing stages, which are shown in **Figure 1.2.A**, to inhibit dough shrinkage during the cutting step. Also, a layering section can be included during the laminating process, where the dough is folded at 45 or 90° and successive layers are laid, thus the biscuit with a flaky structure can be obtained after baking (Tiefenbacher, 2017; Manley, 2011). Examples of these biscuits are *saltine crackers* (*e.g. Crackelet* by Carozzi Costa), *water biscuit* (*e.g. Agua* by Nestlé McKay) and *semi-sweet biscuit* (*e.g. Vino* by Nestlé McKay), which are represented as blue, cyan, and pink points in **Figure 1.1**, respectively.

*Wire-cut, sugar-snap,* and *rotary-moulded biscuits* are prepared from short doughs, which differentiate from all the other categories because they have the highest amount of sugar and fat, the lowest amount of water, and they are prepared using soft flour (9-11% of total protein) (Kweon et al., 2014). Wire-cut and sugar-snap are the most popular among the sweet-biscuits group, so that research on biscuit formulation and manufacturing has been mainly focused on them. The levels of sugar and fat in these biscuits range between 18-35% (d.b.) and 10-23% (d.b.) (Manley, 2011), respectively, and they are illustrated in red and orange points in **Figure 1.1**. Examples of wire-cut and sugar-snap biscuits are *Chips Ahoy!* (by Mondelēz International Nabisco) and *Oreo* (by Mondelēz International Nabisco), respectively. Wire-cut dough is soft, sticky, and sometimes may contain coarse inclusions (*e.g.* nuts, dehydrated fruits and chocolate chips), so that the wire-cutting moulding technology is preferable (**Figure 1.2.B**). Sugar-snap dough is a kind of rotary-moulded dough but, unlike wire-cut, the dough is not that soft and is cohesive enough to be pressed into molds to obtain a moulded dough, as it is exemplified in **Figure 1.2.C**. Depending on the ratio sugar:fat:flour, sugar-snap and rotary-moulded biscuits can share a region in the formulation, as shown in the red area in **Figure 1.1**. However, there is another group of rotary-moulded biscuits that is elaborated with lower amounts of sugar (12-20%, d.b.) and fat (7-12%, d.b.) (green region in **Figure 1.1**), and it accounts for about 70% of market sells in Latin America (Nestlé Information). Examples of these rotary-moulded biscuits are *Morocha* and *Tritón* (by Nestlé McKay). The accumulated knowledge for this group of rotary-moulded biscuits has been limited; consequently, current scientific knowledge cannot be directly applied to this specific biscuit category since its formulation contains less sugar and fat.

This thesis will exclusively focus on rotary-moulded biscuits to get a better comprehension about how the reformulation (to move to healthier recipes) and processing conditions impact their quality attributes.

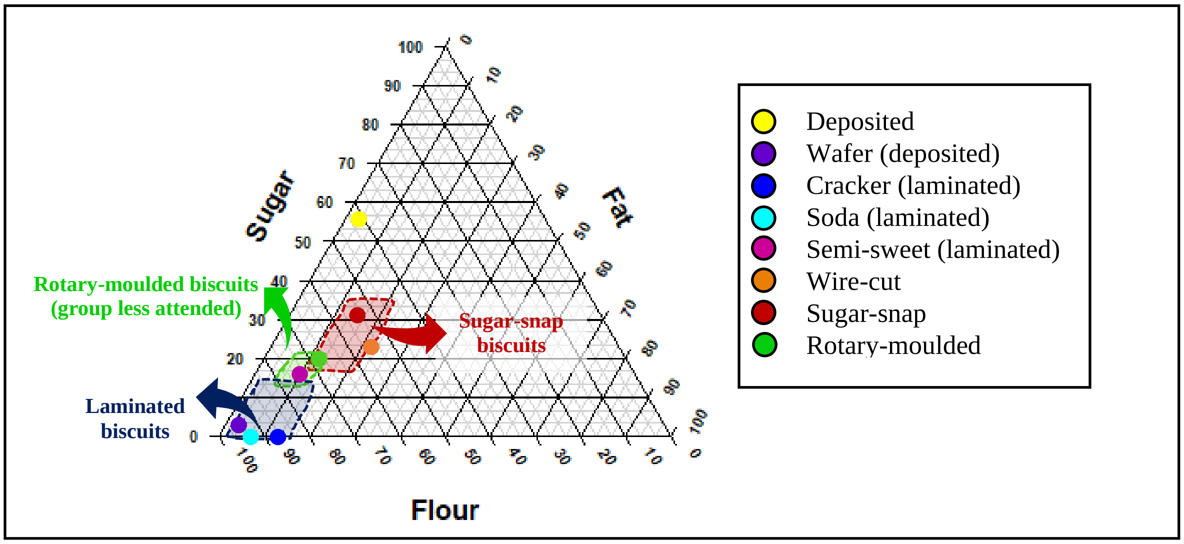


**Figure 1.1** Formulation of biscuits according to the ratio sugar:fat:flour (dray basis of dough) (Obtained from Manley, 2011, and AACC methods: 10.50 and 10.53).

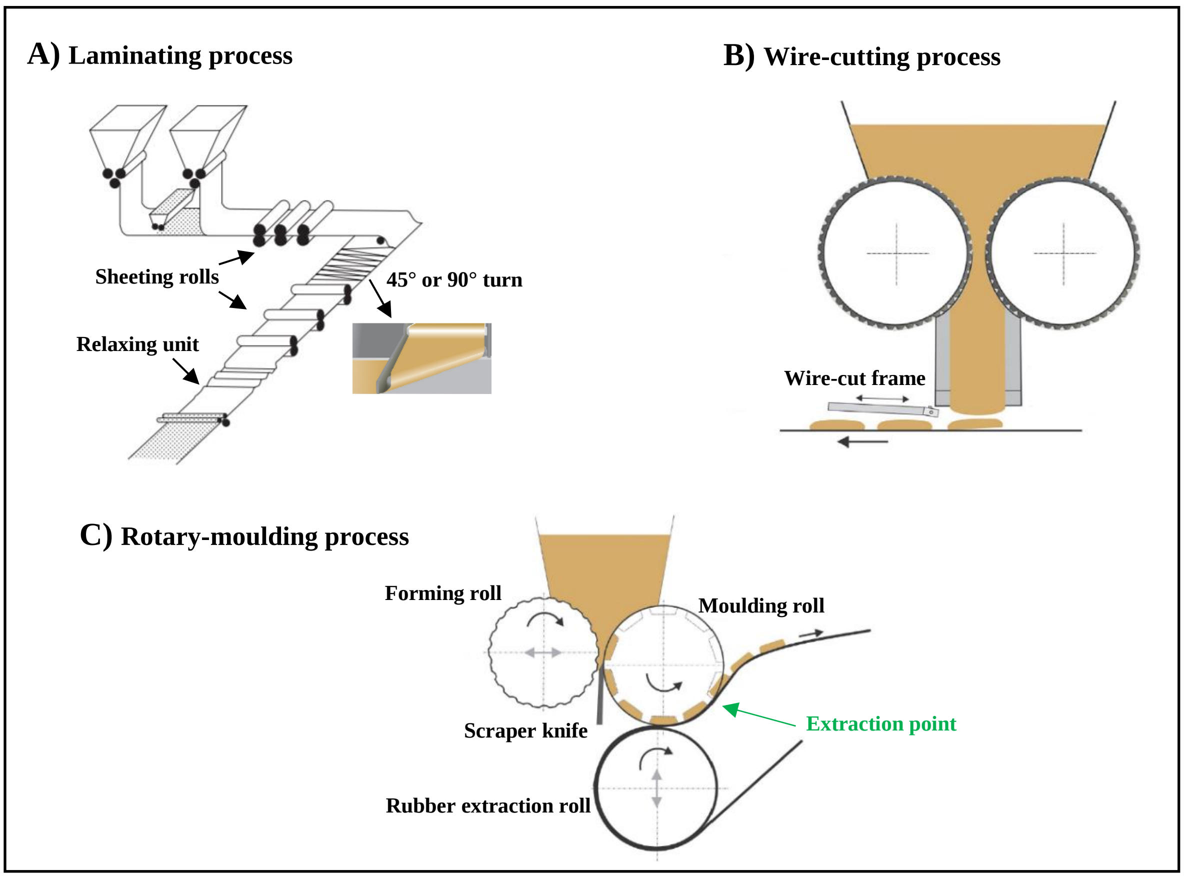


**Figure 1.2** Methods of biscuit dough forming: A) Laminating, B) Wire-cutting, and C) Rotary-moulding (Based on Manley, 2011, and Davidson, 2018).

## 1.3 Rotary-moulding process and the importance of mixing in short dough

The rotary-moulding process is a common method to produce biscuit dough pieces. It should be elaborated with a short dough, which means a dough with low extensibility and elasticity (Manley, 2011). Four main stages comprise the rotary-moulding process: two mixing steps, the creaming (or cream-up) and the dough-up phases, the moulding step, and the baking process, as shown in **Figure 1.3**.

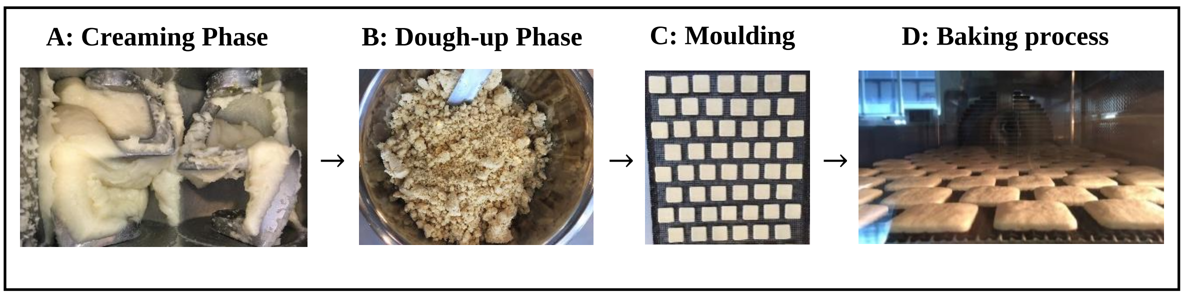


**Figure 1.3.** Overview of the rotary-moulding biscuit process (own elaboration).

The mixing process is a crucial stage in sweet-biscuit-making, because the gluten network formation should be limited before baking. To reducing as much as possible the contact between flour and water, the mixing process is commonly divided into two stages. During the creaming preparation (**Figure 1.3.A**), fat, sucrose, water, leavening agents, and surfactant are blended together during 2 to 5 minutes. It has been stablished that a homogeneous, emulsified and aerated phase should be obtained at the end of this stage (van der Sman & Renzetti, 2018; Manley, 2011; Kamel, 1994). The supposed air incorporated during this phase could act as aeration nuclei for the leavening agents, thus allowing the development of an aerated biscuit structure after baking (Brijwani et al., 2008). During the dough-up (**Figure 1.3.B**), the flour and the remaining leavening agents are added to the creaming phase, at a recommended low mixing speed and in the shortest possible time (no more than 60 to 120 s), to restrict the mechanical work and hindrance gluten development (Kweon et al., 2014). At the end of mixing, a short dough is obtained, which is described by having low elasticity and extensibility because of the minimal formation of a gluten network, as a consequence of the formulation with large amounts of sugar and fat and a low water content (Manley, 2011).

After the short dough is obtained, the dough is moulded using a rotary moulding machine (see **Figure 1.2**). It comprises a dough hopper, where the dough is put inside to feed the forming roll. This roll is also called forcing roll because, once it is filled with the dough, it presses the dough against the moulding roll. The excess of dough is removed by the scraper knife which acts on the surface of the moulding roller. The dough is maintained into the moulding roll until it passes to the point where the extraction is reached, which is achieved by the rubber extraction roll. The tension of the extraction web is controlled by this roller when its position is adjusted up or down. Also, for effective adhesion of the dough piece, the surface of the extraction web must be sufficiently rough. After the dough piece is transferred from the moulding roll to the extraction web, it is carried onto the oven band or tray to proceed to the baking process (**Figure 1.3.D**).

Chevallier et al. (2000a) describe a short dough as a suspension of proteins and starch granules in a continuous liquid sugar solution, in which lipids are emulsified and constitute the disperse phase (**Figure 1.4**). However, due to the low amount of water used in short doughs, probably not all the sugar crystals are dissolved into water, which constitute the sugar solution, so that part of them remain as crystals at the end of the mixing phase. Both the sugar crystal size and the sugar solution have shown being crucial parameters during sweet-biscuit processing as they influence the dough expansion during baking. Deeper explanation about these parameters will be discussed in the following sections.

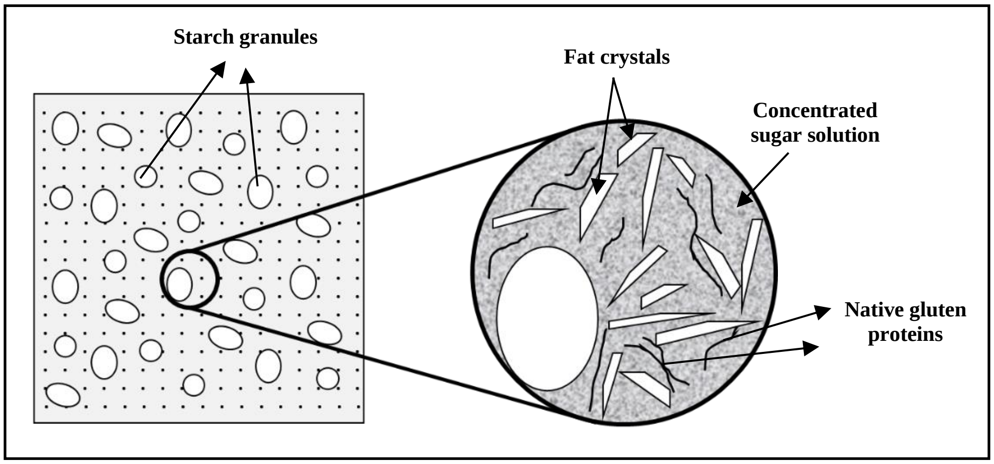


**Figure 1.4** Representation of a short dough, where the creaming phase is illustrated as grey. The location of air bubbles is not pointed out (Based on Chevallier et al., 2000a).

Different mixing methods have been examined to assess the impact of flour hydration on the quality attributes of biscuits. Manohar & Rao (1999) observed that the elastic recovery and cohesiveness of a rotary-moulded dough increased when all the ingredients were blended at once during mixing (all-in-one method), instead of into two stages (with creaming step). These rheological responses negatively affected the quality attributes, and harder and thicker biscuits were obtained, attributing this behavior to the gluten development, because the water availability for flour hydration was enhanced in an all-in-one method. Regarding the mixing time and speed, Manohar & Rao (1997) prepared a rotary-moulded dough by raising the mixing time of the dough-up phase from 90 to 300 s. Despite the fact that biscuit dimensions were not altered, the density and the breaking strength significantly increased in biscuits whose dough was subjected to the largest mixing time. They explained this was a result of an excessive gluten development during the dough-up phase. Contamine et al. (1995) studied the effect of mixing speed during the dough-up phase on the dough viscoelastic properties and quality attributes of semi-sweet biscuits. They showed that shorter and thicker biscuits were obtained as a result of increasing the mixing speed and time. As can be observed, most of the studies have focused on the effect of mixing parameters during the dough-up phase and their impact on the quality attributes of biscuits. However, it is still necessary to understand the impact of mixing parameters on the stability of the creaming phase, and little attention has been put on whether an unstable creaming phase would actually have negative effects on the final attributes of biscuits.

## 1.3 The role of flour, fat and sugar during biscuit-making process

Flour, fat, and sugar are the most important ingredients in biscuit formulation, because each one contributes to the structure development during the biscuit-making process.

### 1.3.1 Flour

Soft wheat flour is the main ingredient used in sweet-biscuit formulation, which is why it is relevant to understand the effect of its components on the rheological behavior of the dough throughout the biscuit-making process. Wheat grain is constituted by wheat bran (~12-16 wt%), endosperm (~80-85 wt%) and germ (~2-3 wt%). After the milling process, the wheat bran and the germ are mostly separated from the endosperm to obtain a soft flour, whose composition is presented in **Figure 1.5**.

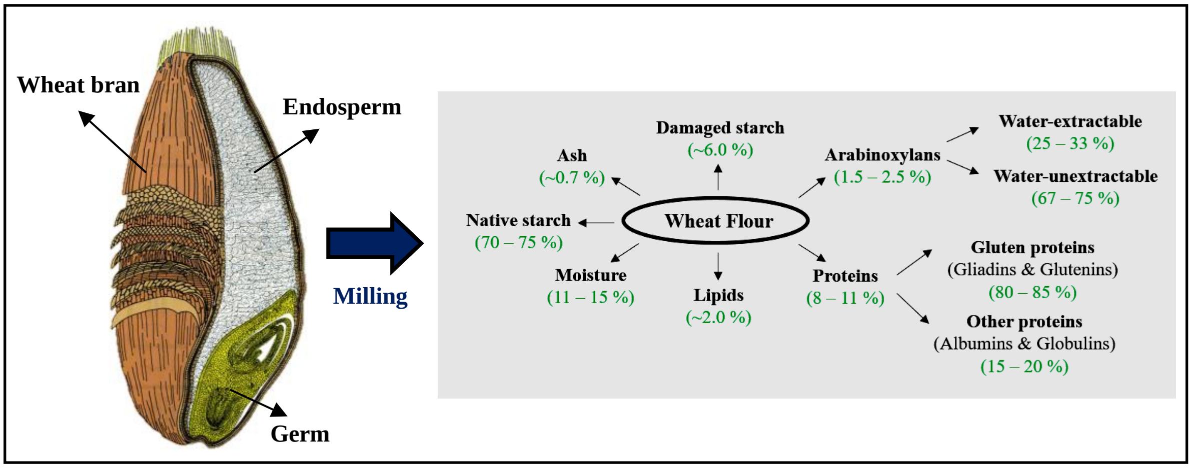


**Figure 1.5** Constituents of wheat grain and composition of soft flour after milling, expressed as weight percent (Based on Onipe et al., 2015, and Manley, 2011).

The major components that influence the variability of flour functionality are arabinoxylans, gluten proteins and damaged starch. Due to their water holding capacity (see **Table 1.1**), these contribute to the flour hydration, and therefore to gluten development.

**Table 1.1** Water holding capacity of flour's components (Based on Kweon et al., 2014, Kweon et al., 2011, and Van Craeyveld, 2009).

| Component | g water/g dry component |
|---|---|
| Gluten | 2.8 |
| Damaged starch | 1.5 – 10 |
| Native starch | 0.3 – 0.45 |
| Gelatinized starch | > 10 |
| Water-extractable Arabinoxylans | 4 – 6 |
| Water-unextractable Arabinoxylans | 7 – 10 |

*Gluten proteins* are gliadins and glutenins, and they constitute between 80 and 85% of the total protein content in soft flour. Gliadins are monomeric proteins with low molecular weight (30 to 80 kDa), which are responsible for dough viscosity and extensibility. Glutenins can be monomeric or polymeric proteins with molecular weights between 12 to 130 kDa, and contribute to dough elasticity (Goesaert et al., 2005; Damodaran, 1996). During mixing, the flour hydration and mixing time are key factors to promote the development of the gluten network (Gupta et al.,1993; Kuktaite et al., 2005). This is formed through the polymerization between gliadins and glutenins, as can be seen in **Figure 1.6**, where intra and intermolecular interactions occur by mainly oxidizing the sulfhydryl groups and sulfhydryl-disulfide interchanges (Létand et al., 1999; Pareyt et al., 2010a). However, it has been mentioned that the gluten development should be inhibited as much as possible because it has a detrimental effect on the quality attributes of sweet biscuits.

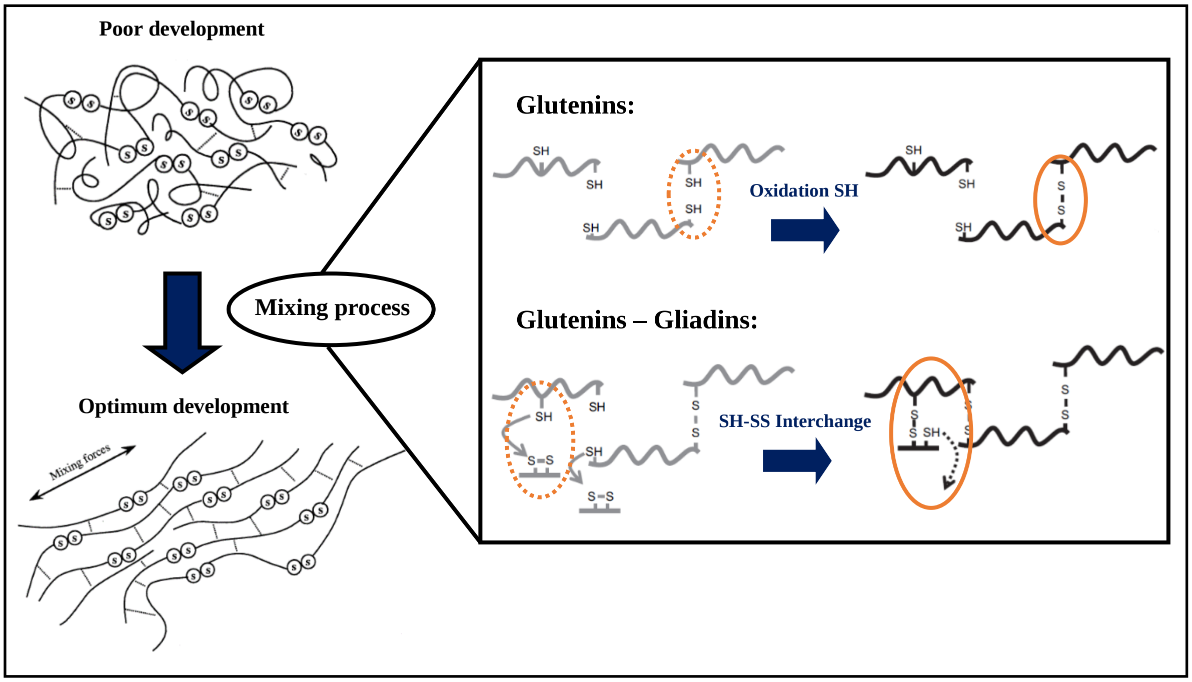


**Figure 1.6** Gluten development during mixing process, and interactions between gliadins and glutenins (Based on Létand et al., 1999, and Pareyt et al., 2010a).

*Wheat starch* is one of the most common carbohydrates present in food. It has a semi-crystalline structure formed by amylose and amylopectin. While amylose is a linear polymer of α-1,4-linked glucoses, amylopectin is a ramified polymer of α-1,4- and α-1,6-linked glucoses (Shannon & Garwood, 2009). These polymers are organized in concentric layers inside the starch granule, where crystalline zones are mainly formed by amylopectin, and the amorphous zones by amylose (Aguilera, 2012). When starch granules are heated in excess of water, at a specific temperature (depending on the botanical source) they start suffering irreversible transformations in their structure. This unleash the gelatinization process (see **Figure 1.7**), which usually occurs between 58 and 64°C in the case of wheat starch (Biliaderis, 2009). The granules lose their molecular order and crystallinity; they swell as a result of water absorption; and, amylose leaches into the aqueous medium, thus increasing the medium viscosity (Colonna & Buleon, 2010; Biliaderis, 2009). This process is dependent on temperature and water availability. This means that, when water availability is insufficient, the gelatinization process is not completed, and the granules will not be 100% swollen. It is important to mention this because sweet biscuit doughs do not have enough water to gelatinize all the starch granules contained in wheat flour, so that partially or even ungelatinized starch has been found in biscuits after baking (Canalis et al., 2018; Laguna et al., 2013a; Pareyt et al., 2008a; Slade & Levine, 1994).

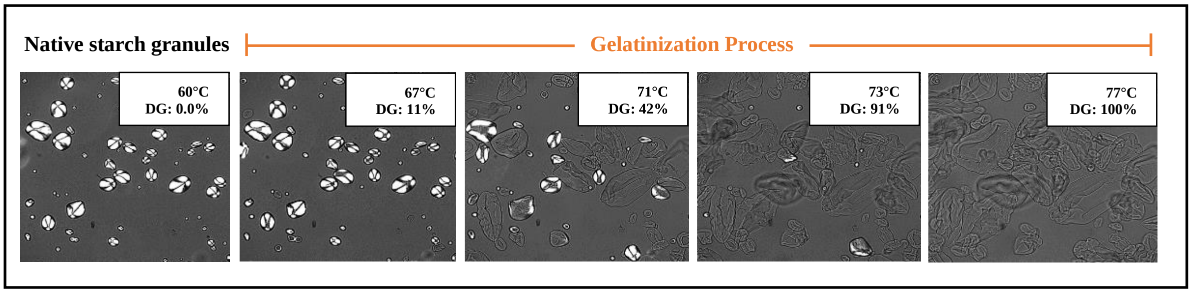


**Figure 1.7** Gelatinization process of potato starch granules immersed into water, observed through hot-stage polarized-light microscopy (heating rate: 15°C/min). DG means degree of gelatinization (Based on Molina et al., 2014).

*Undamaged starch* granules are insoluble in water at room temperature, but they can absorb between 0.3 to 0.45 grams of water per gram of dry starch (see **Table 1.1**). However, during milling of wheat grain, it is common that part of starch granules are physically damaged as a consequence of compression and shearing forces. *Damaged starch* can absorb more water at room temperature, from 1.5 to 10 grams of water per gram of dry starch depending on the damaging level (**Table 1.1**). Studies have shown that damaged starch significantly increases the water holding capacity of flour (Barrera et al., 2013; Barak et al., 2012; Kweon et al., 2011; Leon et al., 2006). This is undesirable in biscuit-making because it negatively affects the dough expansion during baking, decreases the biscuit dimensions and increases the hardness, as it has been presented by Barrera et al. (2007) and Barak et al. (2012).

*Arabinoxylans* are non-starch polysaccharides that constitute between 1.5 and 2.5 wt% of soft flour, and they are divided into water-extractable and water-unextractable. Arabinoxylans are the most important fiber comprising the cell walls of the wheat grain, especially in wheat bran (70% arabinoxylans, 24% cellulose, and 6% β-glucans (Maes and Delcour, 2002), as it can be observed in light green in **Figure 1.8**.

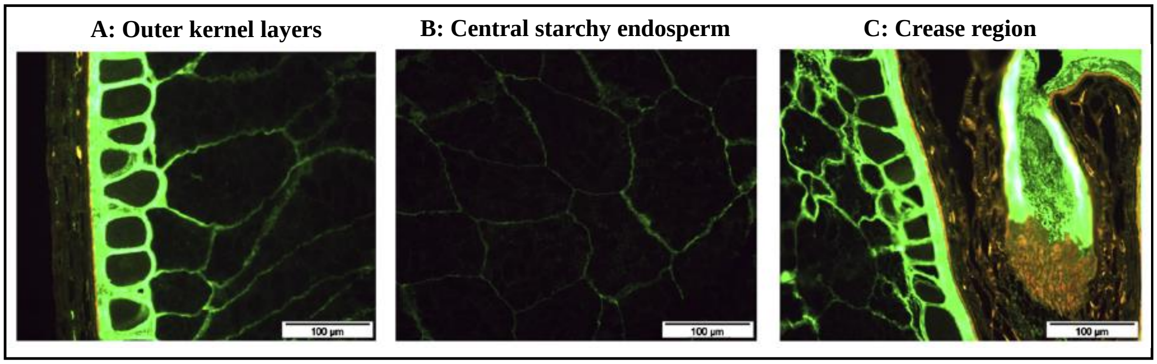


**Figure 1.8** Cross sections (A: outer kernel layers; B: central starchy endosperm; C: crease region) of a wheat kernel stained with *Alexa Fluor®488-labeled catalytically inactive Bacillus subtilis xylanase* (Taken from Dornez et al., 2011).

Despite of arabinoxylans being found in low proportion in soft flour, they have the highest water holding capacity among the other flour components (see **Table 1.1**), so that it is important to understand their contribution to the structure of biscuits. Most of the scientific knowledge about the effect of soft flour arabinoxylans on biscuits has been taken from correlations between biscuit dimensions and the solvent retention capacity test (SRC test). The SRC is a solvation test for flours to study the functional contribution of glutenins, damaged starch and water-extractable arabinoxylans to the flour-swelling behavior (Kweon et al., 2011). Pasha et al. (2009), Colombo et al. (2008) and Ram & Singh (2004) detected a positive correlation between the SRC test (for arabinoxylans) and the thickness of sugar-snap biscuits. In contrast, Duyvejonck et al. (2011) examined the contribution of flour constituents to the SRC profile for nineteen European wheat flours, and they did not observe any relationship between SRC values and either total, water-extractable or water-unextractable arabinoxylans.

Whole wheat flour incorporation can be a convenient and non-expensive alternative to enrich biscuits with bran fiber. Wheat bran contains about 46% total weight of dietary fiber and it is the main by-product of milling, which is mostly used for animal feed (Onipe et al., 2015). It has been shown that bran reduces the specific volume in bread and saltine-cracker biscuits, because its particles interfere the formation of the gluten network (Lapčíková et al., 2019; Le Bleis et al., 2015; Li et al., 2014). However, the formulation of sweet biscuit doughs is quite different from bread or saltine-cracker doughs, as it was shown in **Figure 1.1**.

Although the gluten network is restrained in these sweet doughs, bran enrichment may have an effect on the rheological properties and baking performance of the biscuit dough, thus impacting final biscuits attributes. Nevertheless, there is still necessary to examine the specific contribution of arabinoxylans, compared to their incorporation entrapped into wheat bran, during processing of rotary-moulded biscuits.

### 1.3.2 Fat

Fat influences the overall texture of biscuits because it provides tenderness, mouthfeel and a lubricating effect during chewing. Among the physicochemical properties, the solid fat index (SFI) is considered the main criteria to evaluate the functionality of fat in biscuits (Devi & Khatkar, 2016). This is defined as the ratio of solid to liquid fat at a given temperature, which depends on the composition of the fatty acids (Ghotra et al., 2002), as it is shown in **Table 1.2**. It has been stablished by biscuit manufacturers that the presence of some solid fat (~20%) is important for the aeration of the creaming phase during mixing (Manley, 2011). Fat crystals adsorb to the surface of air bubbles, thus stabilizing the aerated structure of the creaming phase (Chevallier et al., 2000a; Brooker, 1993). Furthermore, fat contributes to the lubrication of flour particles, so that it limits the contact between flour and water, and a thinner and larger biscuit can be obtained (Pareyt & Delcour, 2008a; Maache-Rezzoug et al., 1998). However, the growing concern about the high levels of saturated fatty acids (SFAs) on baked products, has promoted the partial or total replacement of fat by unsaturated vegetable oils. It has shown that the higher proportion of unsaturated fatty acids presented in these oils (see **Table 1.2**) decreases the LDL-cholesterol production (Siri-Tarino et al., 2010). Studies have seen that oil replacement increases the breaking strength of sugar-snap and rotary-moulded biscuits, which detrimentally affects the final texture (Devi & Khatkar, 2018; Tarancón et al., 2015; Jacob & Leelavathi, 2007). Nevertheless, emulsifiers have been used to modulate the biscuit hardness when oil is incorporated to the formulation of sugar-snap biscuit as a replacement of fat (Jacob & Leelavathi, 2007).

**Table 1.2** Physicochemical properties of some fats and oils (Based on Devi & Khatkar, 2016; Akhtar et al., 2014; O'Brien, 2008).

| Property | Palm Oil | Coconut Oil | Peanut Oil | Sunflower Oil | Cottonseed Oil |
|---|---|---|---|---|---|
| Solid Fat Index (SFI, %) at 21.1°C | 14.0 | 26.6 | -- | -- | -- |
| Melting Point (°C) | 37.5 | 25.5 | 3 | -17 | 13 |
| Saturated fatty acids (SFAs, %) | 50.4 | 92.0 | 18.4 | 12.4 | 25.7 |
| Unsaturated fatty acids (UFAs, %) | 49.6 | 8.0 | 80.3 | 87.6 | 74.3 |

### 1.3.3 Sugar

Sucrose is the most important sugar in biscuit manufacturing. In addition to the sweetness function, it provides structure and texture properties to biscuits by modifying the thermal/viscoelastic behavior during biscuit-making (Chiotelli et al., 2000). Sugars can be added to the dough formulation as crystal or syrup, so that the particle size distribution and solubility in water have shown being crucial parameters during biscuit processing (Canalis et al., 2018; Pareyt et al., 2009b; Kweon et al., 2009; Doescher & Hoseney, 1985). The rate of sucrose dissolution in water depends on its particle size, which has shown being critical in sugar-snap and wire-cut doughs with high sucrose concentration, because it influences the dough expansion during baking (Slade & Levine, 1994). **Figure 1.9** illustrates the changes of sugar-snap biscuit dimensions as a result of the sugar particle size and its dissolution into water. While finer sucrose particles dissolve faster in water thus promoting the lateral spread of dough and producing larger and thinner biscuit; for their part, coarser sucrose particles dissolve slower in water, which produces a shorter and thicker biscuit (see **Figure 1.9**) (Kweon et al., 2009).

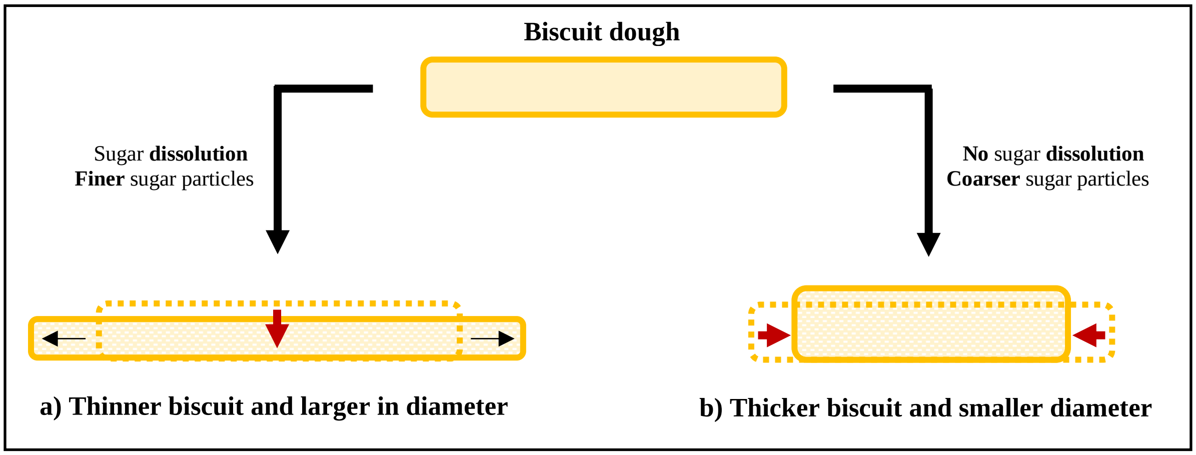


**Figure 1.9** Representation of the effect of sugar particle size and its dissolution into water on the biscuit dimensions after baking (own elaboration, based on Pareyt et al., 2009b, and Kweon et al., 2009).

Dietary concerns about consumer's high sugar intake is driving governments to apply front-of-package (FoP) nutrition labelling policies to educate consumers and make easier their choices (EUFIC, 2018). Biscuit companies have had to reformulate their recipes to reduce the amount of added sugar replacing it with different alternatives, such as polyols, dextrins, and oligosaccharides (hydrocolloids or fibers) (van der Sman & Renzetti, 2018). This has been challenging because these new ingredients should mimic sugar functionality, such as the sweetness, the effect on thermal properties of starch granules and gluten proteins, the non-enzymatic browning, and the sensory attributes (Struck et al., 2014). Studies have shown that, in sugar-snap doughs, 50% of sucrose reduction promotes starch gelatinization and gluten entanglement. As a result of this, the spread of dough becomes lower during baking, and the hardness of biscuit increases due to a less aerated structure (Canalis et al., 2018; Laguna et al., 2013a). Therefore, it is necessary to have a better comprehension of the functionality of sucrose in rotary-moulded biscuits, to modulate the structure with a lower sugar content and hopefully without FoP nutrition labels.

## 1.4 Physicochemical changes of dough during baking

The biscuit baking process can be carried out in static or continuous ovens and, depending on their characteristics, the heat can be transferred to the dough by conduction, convection and/or radiation. The main advantage of using continuous ovens is that the temperatures and the heat transfer conditions can be adjusted throughout the oven (Manley, 2011), which is why they are generally divided into 3 to 5 baking zones. Three major changes can be identified during biscuit dough baking as shown in **Figure 1.10**, which are structure development, dehydration (*i.e.* vigorous water loss) and browning (*i.e.* surface coloration).

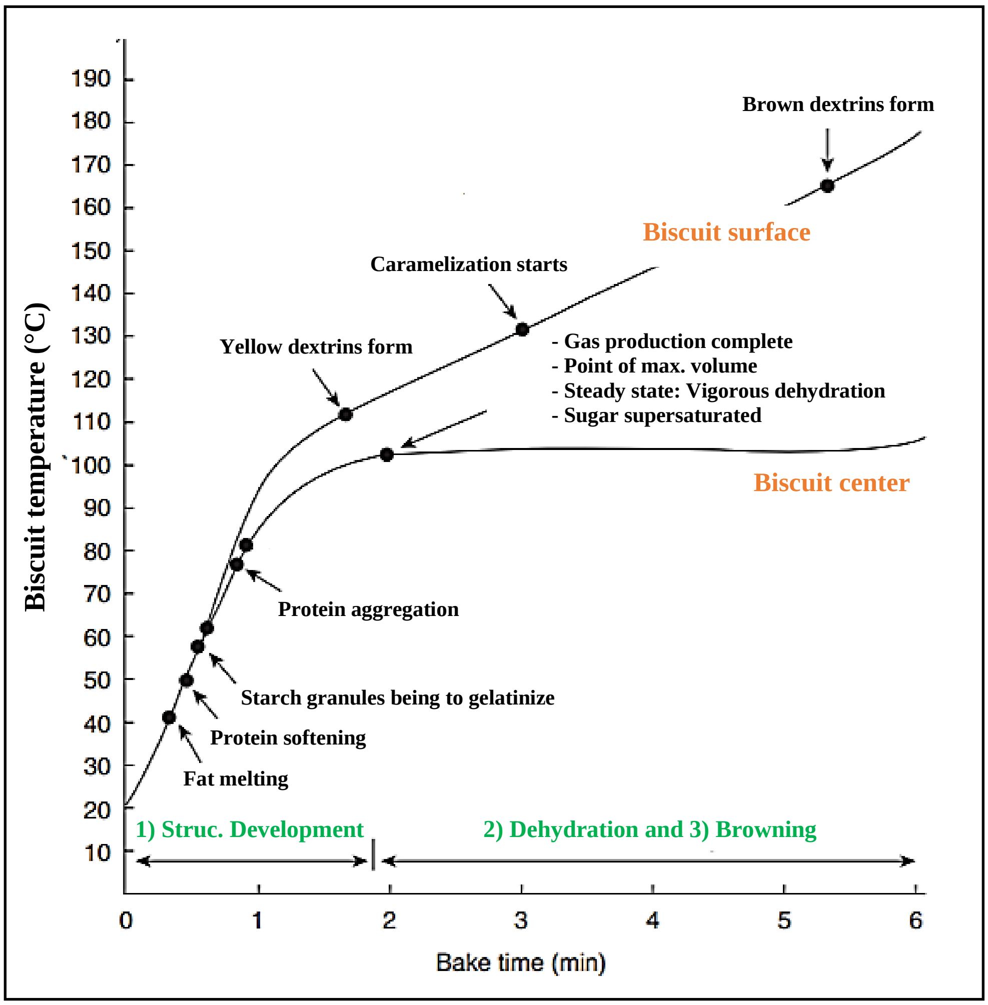


**Figure 1.10** Changes of biscuit dough during baking (Taken from Manley, 2011).

The *structure development* takes place during the first third of the baking process, where the temperature of the dough rapidly increases up to 100°C. Baking parameters such as oven temperature, air velocity, and the humidity ratio of air (g of water per kg of dry air) affect the dough temperature, the dough expansion, the water loss, the dough coloration, and the formation of a crust on the surface of biscuit dough, as can be observed in **Figures 1.11.A-D**. Because of the low humidity ratio of air and/or the high temperature, the biscuit dough partially dehydrates during this first stage, as shown in **Figure 1.11.B**, which may promote an early crust formation impacting the structure development of biscuit dough. At 40-45°C the fat thermal transition occurs, and an oily phase is obtained, which helps to increase the mobility of dough (Pareyt et al., 2009b). Sugar crystals also dissolve during this stage, which may impart mobility to the dough because of the greater solution volume as a result of sugar dissolution (Davis, 1995). Increased mobility allows the dough to laterally spreads and vertically expands, where vertical expansion is more pronounced than horizontal spread mainly due to the effect of leavening agents. **Figure 1.11.C** shows the vertical expansion of biscuit doughs subjected to different baking conditions. This dimension may increase up to 4 times the original thickness until achieving a maximum point, where the expansion process is completed because of an increment in the viscosity that prevents further expansion (Chevallier et al., 2002; Doescher et al., 1987a). A semi-rigid structure is needed to control and withstand the lateral spread and the vertical expansion due to the steam pressure and the carbon dioxide produced by chemical leaveners. Controlling the dough expansion of sugar-snap biscuits has been mainly attributed to gluten proteins denaturation, which become insoluble as aggregation/cross-linking occurs above 85°C (Pareyt et al., 2008b; Chevallier et al., 2002). However, there is no consensus yet about whether the gelatinization process takes place during biscuit baking, and thus whether it is responsible for limiting the dough expansion. While Laguna et al. (2013a) and Chevallier et al. (2000a) showed that starch granules partially gelatinized (< 50%) in sugar-snap doughs with 21 or 26% (d.b.) of added sugar; Pareyt et al. (2009a) and Pareyt et al. (2008b) concluded that no gelatinization occurred in sugar-snap doughs with 25 to 35% (d.b.) of added sugar, and that gluten is the main responsible for restricting the dough expansion during baking.

The *dehydration* constitutes the second stage during baking. Although part of the water vapour migrates from the biscuit dough during the structure development stage, the vigorous water removal takes place at the surface of biscuit dough when it achieves the boiling temperature, and it occurs as the biscuit dough is near or on the maximum expansion. Additionally, some extraction hoods are partially opened to remove the moist air from the oven, which is replaced by hot and dry air to increase the driving force of drying. **Figure 1.11.B** shows an improvement in the kinetic of water loss when increasing the baking temperature and the air velocity. At the same time as dehydration occurs, the concentration of dissolved sugar increases, producing a viscous and supersaturated solution (van der Sman & Renzetti, 2018; Slade & Levine, 1994). Furthermore, the biscuit dough also undergoes a vertical collapse, as can be seen in **Figure 1.11.C**, and it is suggested that collapse occurs because the dough has not become rigid enough to overcome the effect of gas loss and to support its own weight under gravitational stress (van der Sman & Renzetti, 2018; Chevallier et al., 2002; Slade & Levine, 1994). As a result of drying, the weight and the density of biscuit dough largely decreased, and a porous structure is developed (Chevallier et al., 2002).

During the last stage of baking, *non-enzymatic browning reactions* occur in the biscuit from 100°C. The Maillard reaction, sugar caramelization, and starch dextrinization are mainly responsible for the yellow-brownish color obtained at the end of this stage (Manley, 2011, Chevallier et al., 2000a). **Figure 1.11.D** illustrates the change of the lightness value (L* channel) at the biscuit surface, which is detrimentally affected by the baking temperature, as occurs with the dashed line curve (condition #1).

At the end of baking, the final dimensions reflect the magnitude of lateral spread and vertical expansion/collapse during baking. Because of that, the ratio *diameter:thickness* has been taken as the main quality indicator in biscuits, and a greater value is desired because this means that a crispier and thinner biscuit can be obtained (van der Sman & Renzetti, 2018). Biscuits are characterized by having a roasted color, being sweets, aerated, crispy or crunchy, and firms, which define the biscuit's texture and flavor perception during chewing. As a result, a wide list of sensory attributes (see **Table 1.3**) has been analyzed in order to understand the effect of ingredients reformulation on the sensory profile and overall liking of consumers (Tarancon et al., 2014).

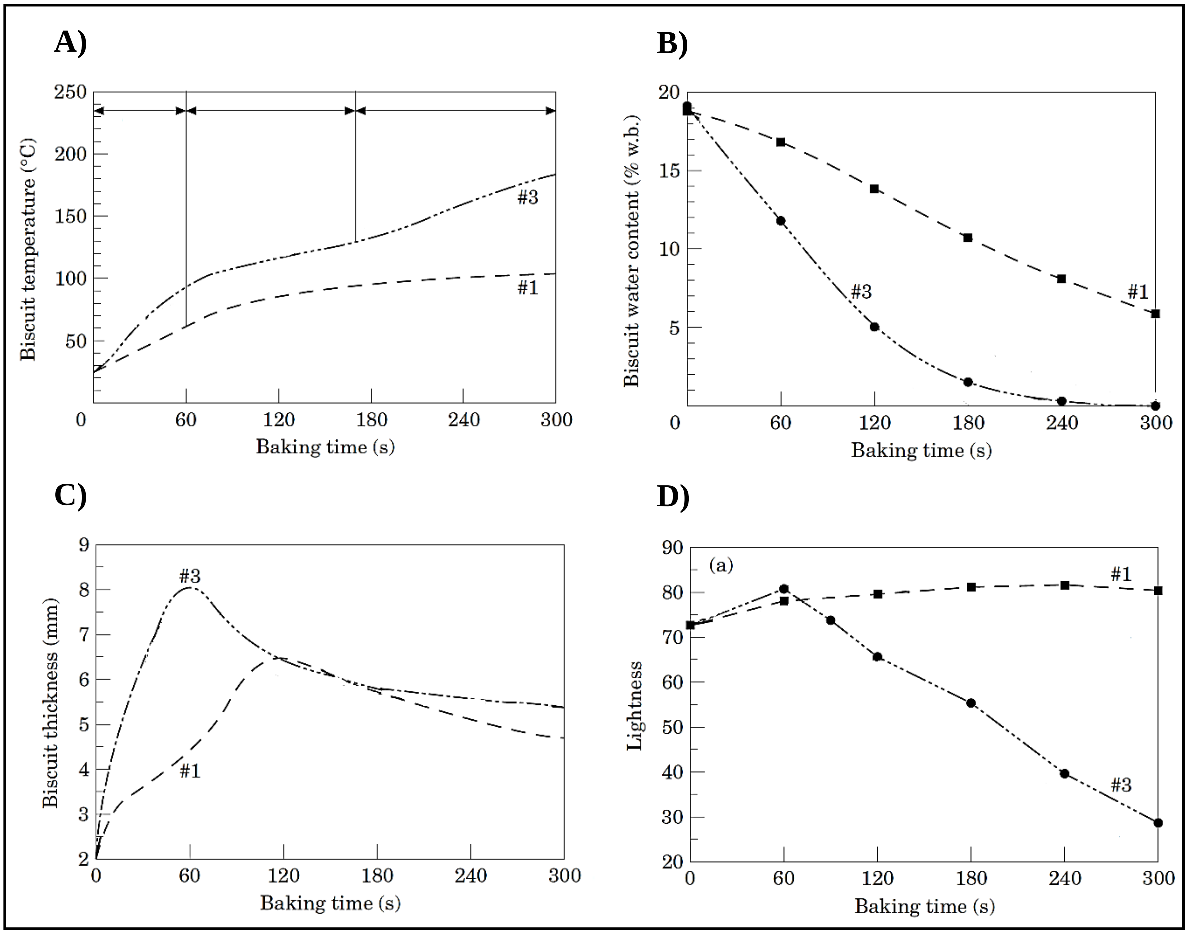


**Figure 1.11** Biscuit temperature (A), biscuit moisture content (B), biscuit thickness (C), and biscuit lightness (D) as a function of the baking time at different baking conditions: #1: 180°C, 20 g/kg dry air, 13 m/s air and #3: 300°C, 20 g/kg dry air, 21 m/s air. Thermocouples were located at the center of the biscuit dough (Taken from Chevallier et al., 2002).

**Table 1.3** Descriptive attributes for sensory profiling in evaluation of biscuits (Based on Le Calvé et al., 2019; Yilmaz et al., 2015; Laguna et al.¸ 2013a).

| Attribute | Definitions |
|---|---|
| **Appearance** | |
| Aeration | Visible air pores (number and size) at the cross section of the biscuit. |
| Color | Level roasted color at the surface of the biscuit. |
| **Texture** | |
| Grittiness | The presence of small dry particles which tend to scrape off the tongue. |
| Hardness | Force required to break the biscuit after the first bite with incisors. |
| Crispiness | High pitched sound produced when the teeth crack the product during mastication, as in a potato crisp. |
| Crunchiness | Low pitched sound produced on biscuit fracture during mastication, as in an almond. |
| Crumbliness | Disintegration into crumbs at the first bite. |
| Dry mouthfeel | Feeling of dryness in the mouth. |
| Fat mouthfeel | Feeling of film fat or oil in mouth. |
| **Taste and aroma** | |
| Overall taste & aroma | Taste and aroma global perception when having a piece of biscuit in mouth. |
| Sweetness | Level of perceived sweetness during chewing of biscuit. |
| Cereal | Cooked cereals. |

## 1.5 Characterization of sweet doughs and biscuit development during baking

The physicochemical changes in biscuit dough structure during baking, such as, dough expansion, starch gelatinization, and gluten proteins denaturation, have been analyzed thorough time-lapse photography (Chevallier et al., 2002; Doescher et al., 1987b), differential scanning calorimetry (Canalis et al., 2018; Chevallier et al., 2000b), and high-performance liquid calorimetry (Pareyt et al., 2009b; Pareyt et al., 2008b). In addition, the biscuit microstructure has been visualized by scanning electron microscopy (Mamat & Hill, 2014; Rodríguez-García et al., 2013) and X-ray computed microtomography (Yang et al., 2012; Frisullo et al., 2010; Pareyt et al., 2009a). Most of these techniques will be used in this thesis to understand the effect of ingredients reformulation during processing of rotary-moulded biscuits.

### 1.5.1 Time-lapse photography during baking

Time-lapse photographic analysis has been widely used to monitor the dough expansion during baking (Pareyt et al., 2008b; Chevallier et al., 2002; Doescher et al., 1987b). This technique consists in recording the baking process with a digital camera or taking images at certain time intervals. To transform the qualitative material into quantitative information, it is necessary to perform an image analysis processing, where the calibration step is key to achieve a proper measurement from digital images. As a result, information about the evolution of the dough expansion as a function of baking time can successfully be extracted from digital images, as shown in **Figure 1.12**.

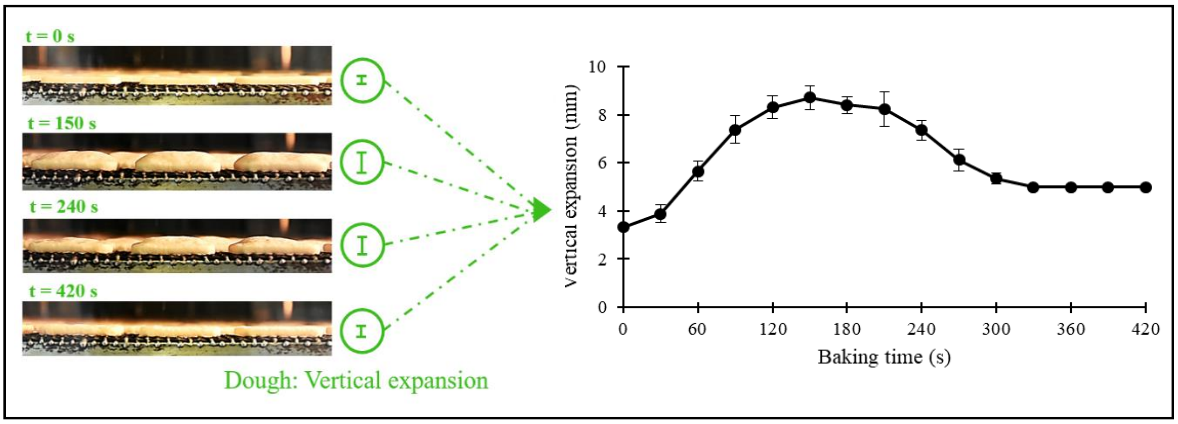


**Figure 1.12** Vertical dough expansion, expressed as a difference of the initial height and the height at specific time of baking, monitored by lapse-time photography (own elaboration).

### 1.5.2 Starch gelatinization by differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique that measures the heat changes of materials as a function of temperature, which are associated to physical/chemical endothermic or exothermic processes (Biliaderis, 1983). DSC has been used to analyze the phase transitions of starch during heating of biscuit dough, as well as the effect of sugars, fats and fibers on this phenomenon (Canalis et al., 2019; Laguna et al., 2011; Chevallier et al., 2000b; Slade & Levine, 1994; Abboud & Hoseney, 1984). One way to measure the gelatinized starch in biscuit during baking is subjecting the dough, and its corresponding baked biscuit, to heating through DSC in excess of water (Contardo et al., 2016) (see **Figure 1.13**). The enthalpy value of the dough, $\Delta H_{dough}$, is obtained after heating the raw dough in the DSC and therefore corresponds to the gelatinization enthalpy of all the starch granules that were embedded in the dough, which were forced to gelatinize in excess of water. On the other hand, $\Delta H_{biscuit}$ represents the enthalpy of the non-gelatinized starch that remained in the biscuit after baking, and is obtained after heating the baked biscuit in the DSC, also, in excess of water. Consequently, the difference $\left(\Delta H_{dough} - \Delta H_{biscuit}\right)$ will denote the amount of starch that was able to gelatinize during baking, and the degree of gelatinization (DG, %) can be calculated as shown in **Figure 1.13**.

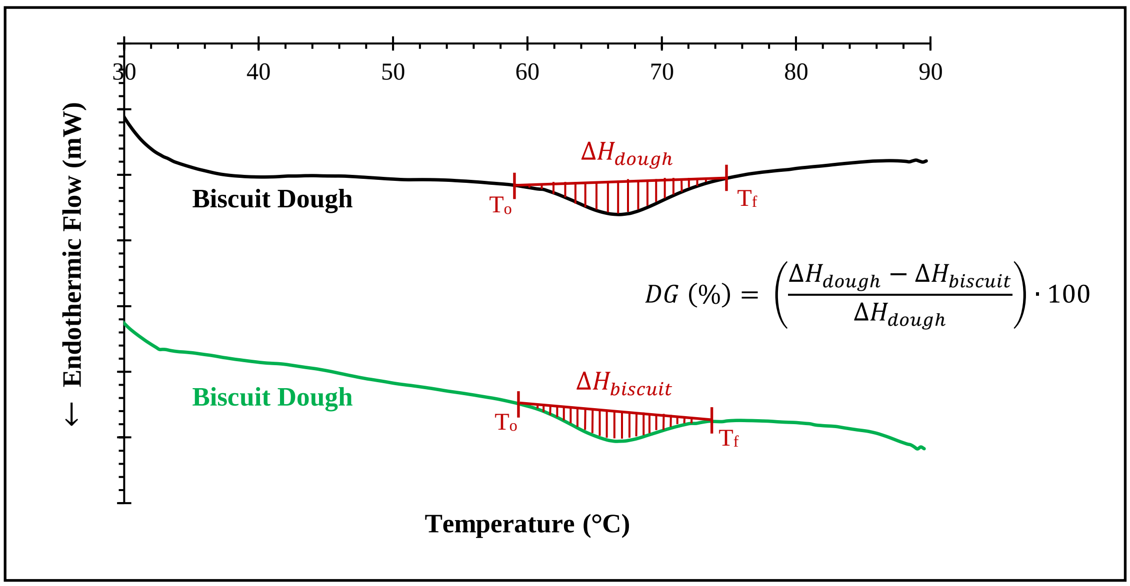


**Figure 1.13** Enthalpy curves obtained by DSC for dough and biscuit with 16.3% of sugar (dough, w.b.). Red area corresponds to the starch gelatinization enthalpies ($\Delta H_{dough}$ or $\Delta H_{biscuit}$), where $T_o$ and $T_f$ are the *onset* and *final* gelatinization temperatures (own elaboration).

### 1.5.3 Biscuit microstructure visualization by scanning electron microscopy

Scanning electron microscopy (SEM) allows the three-dimensional (3D) imaging of the sample microstructure with great resolution (1-5 nm). When a finely focused electron beam impacts the sample, it produces two outgoing signals that are often used to create an SEM image: secondary electrons and backscattered electrons (Goldstein et al., 2018; Aguilera & Bouchon, 2008). Biscuit components such as fat, starch granules and gluten proteins have been visualized through this technique (Mamat & Hill, 2014; Rodríguez-García et al., 2013; Pareyt et al., 2010a). Before the analysis, it is important ensuring the biscuit sample is dry and coated (with conducting material) to obtain a high-quality electron image (see **Figure 1.14**). Hydrated samples cannot adequately be observed under high-vacuum environment because water evaporation occurs, and the sample may suffer serious microstructural changes (e.g. shrinkage) as a consequence of dehydration. Due to this, variable pressure scamming electron microscopes are alternatively used for analyzing wet specimens. The microstructure of rotary-moulded biscuit can effectively be observed under high pressure SEM, because drying occurred during baking and the water content was reduced to value lower than 4% (w.b.), which does not interfere during the analysis. Furthermore, biscuits are usually defatted, as fat may interfere the observation of other biscuit's components. Also, the non-coated biscuit cannot adequately be analyzed at high-vacuum environment because it acts as an electron trap creating extra-white regions that also influence the visualization.

### 1.5.4 Biscuit Microstructure Quantification by X-ray Computed Microtomography

X-ray computed microtomography (X-ray μCT) is a non-destructive and non-invasive technique, which allows not only scanning a sample with good resolution (>1 μm), but also quantifying the internal structure (Schoeman et al. 2016). However, microstructural analysis of biscuits using X-ray μCT is still limited (Yang et al., 2012; Frisullo et al., 2010; Pareyt et al., 2009b), however, it may provide three-dimensional information about air pores and wall thickness that can be instrumental to characterize biscuit structure.

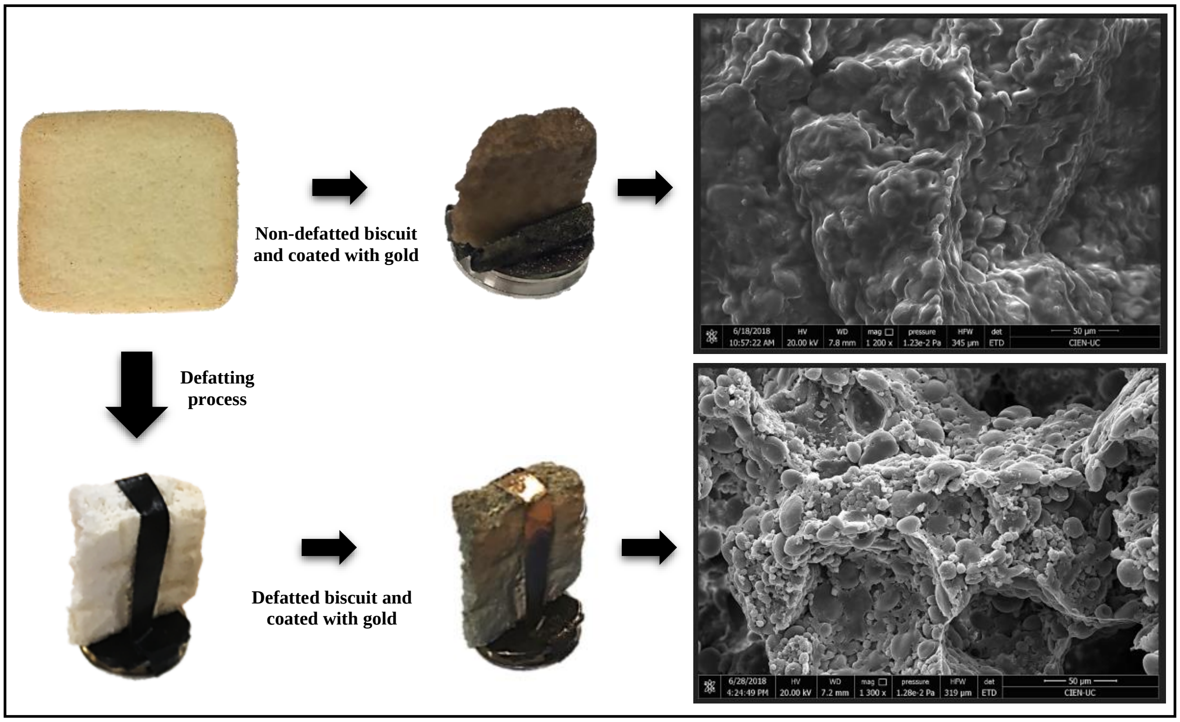


**Figure 1.14** SEM visualization of the biscuit's microstructure before and after subjecting it a defatting process (own elaboration).

The principle of this technique is based on the image contrast produced when the X-ray beam passes through the sample, because X-rays are attenuated mainly due to the material's density and its atomic number (Schoeman et al. 2016). The 2D detector measures the X-rays attenuated and records the image projections at every rotation angle (see **Figure 1.15**), in order to use them later for reconstruction and quantification steps. The reconstructed images need to be processed in order to remove residual noise, to create a region of interest, and in this case, to segment the image to get the biscuit matrix and the air pores, in order to obtain 3D quantitative information associated to these regions.

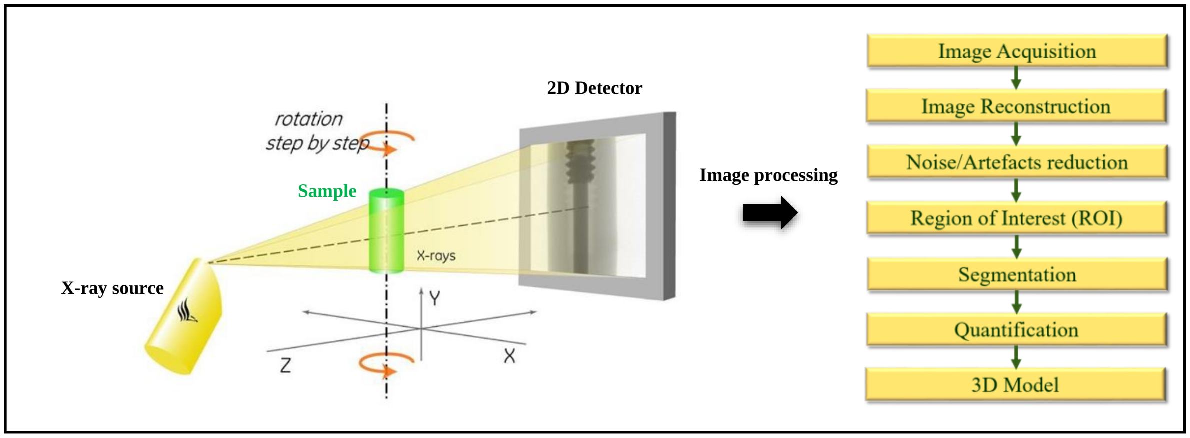


**Figure 1.15** Schematic illustration of the measurement principle of X-ray µCT (From Lv, 2018).

As a result of the image processing, a three-dimensional representation of the biscuit internal walls and the air pores can be obtained, as well as a size distribution of these components, as can be seen in **Figure 1.16**.

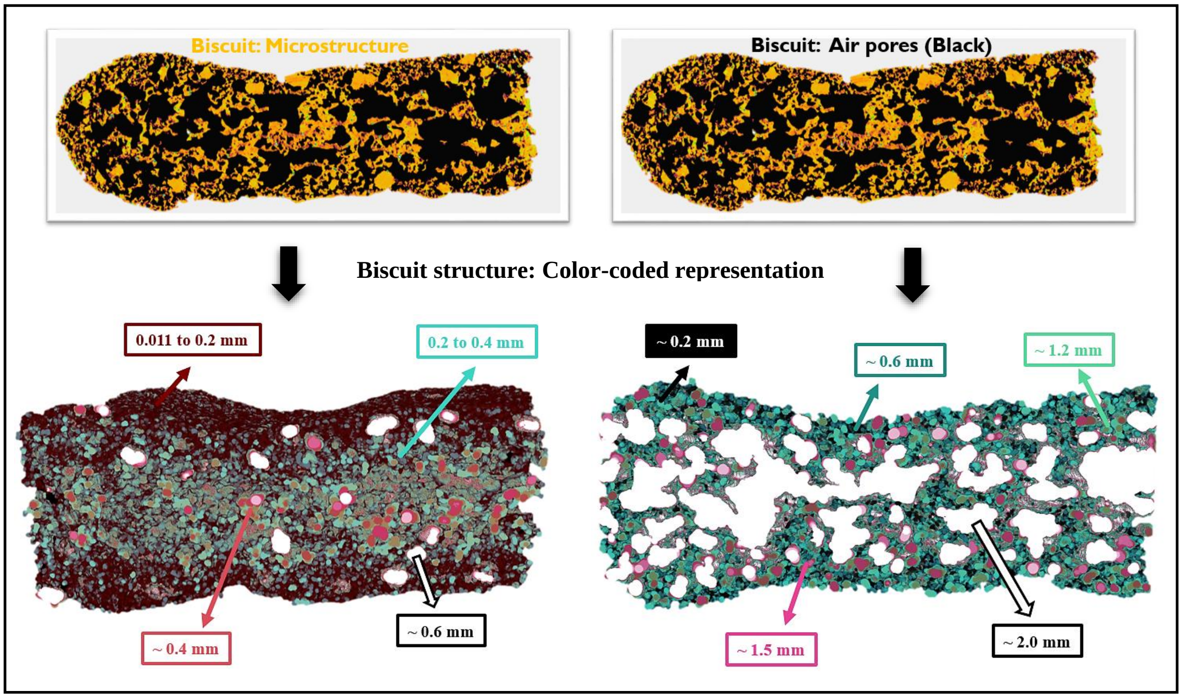


**Figure 1.16** 3D representation of the biscuit's internal walls and air pores, where the size of structures is illustrated by different colors (own elaboration).

## 1.6 Hypothesis and Objectives

Consumers' growing concerns about nutrition and excessive caloric intake has moved the industry to provide healthier biscuits. However, reformulating ingredients to produce fiber-rich or sugar-and-fat-reduced biscuits compromises their texture and flavor. Some key aspects such as processing parameters during mixing and baking, the creaming stability, and the critical thermal transformations for the structure development of biscuits, may help to have a better understanding of the microstructural and sensory-profile modifications of rotary-moulded biscuits when reformulating their ingredients.

The hypothesis of this research is that through the understanding of the effect of creaming stability, sucrose concentration and wheat bran incorporation during the biscuit-making process, it is possible to modulate critical quality attributes, such as aeration level, sweetness perception and hardness, in order to set-up the basis to provide high quality and healthier rotary-moulded biscuits. Specifically, the creaming stability will possibly affect the flour hydration and, consequently, the structure development of dough during baking, altering the sensory profile of biscuits; the sugar reduction may promote starch gelatinization changing microstructural parameters of biscuit such as porosity, air distribution and the thickness of biscuit walls, and the sugar particle size could influence the sugar perception during chewing; finally, the incorporation of wheat bran as an important source of arabinoxylans may modify the dough rheology impacting the microstructure of rotary-moulded biscuits.

In accordance, the main objective of this research is to understand the impact of the creaming stability, the sucrose reduction and the wheat bran enrichment on the structure development during processing of rotary-moulded biscuits, in order to modulate (micro) and (macro) structural quality attributes such as the aeration level, the sweetness perception and the texture hardness. For this purpose, a microstructural approach is conceived to ascertain the link between sensory attributes and biscuit properties at micro and macro-structural levels.

The specific objectives are:

1. To determine whether an unstable or stable creaming phase may modify the quality attributes (*i.e.* dimensions, microstructure, hardness, and sensory profiling) of rotary-moulded biscuits when using different sucrose particle sizes and sugar concentrations when adding a sugar solution.

2. To analyze the influence of sucrose reduction and particle size on dough expansion and starch gelatinization during baking of rotary-moulded biscuits using a microstructural approach, and their link to sweetness perception during oral processing.

3. To assess the effect of water-unextractable and water-extractable wheat arabinoxylans addition, as well as the effect wheat bran flour enrichment, on the dough rheology and starch gelatinization, and on the resultant microstructure of rotary-moulded biscuits.

# OUTLINE OF THE THESIS

The aim of this thesis is to study the impact of the creaming stability, the sucrose reduction and the wheat bran enrichment on the structure development during processing of rotary-moulded biscuits using a microstructural approach to ascertain the link between sensory attributes and biscuit properties at micro and macro-structural levels.

**Chapter 1** presents an overall introduction about biscuit categories and the rotary-moulding process. Also, this chapter includes the effect of flour polymers, sugar and fat during biscuit processing, critical parameters during mixing stage, structural changes of biscuit dough during baking. Moreover, methodologies to analyze the microstructure of biscuit were described, since they were elaborated during the thesis development or adapted from already published research to characterize microstructural parameters such as porosity, air distribution and the thickness of biscuit walls.

**Chapter 2** focuses on analyzing whether an unstable or a stable creaming phase may modify the quality attributes of rotary-moulded biscuits, such as dimensions, microstructure, hardness, and sensory profiling, by using different sucrose particle sizes and concentrations of sugar solution.

**Chapter 3** analyzes the specific contribution of water-unextractable and water-extractable arabinoxylans during biscuit-making, and the relationship between this contribution and the effect on the structure development of sweet biscuits when they are incorporated as wheat bran in the replacement of refined flour by whole flour.

**Chapter 4** discusses the influence of sucrose reduction and sucrose particle size on dough expansion and starch gelatinization during baking of rotary-moulded biscuits using a microstructural approach (X-ray micro-computed tomography and scanning electron microscopy), and their link to sweetness perception during oral processing.

A schematic overview of the thesis is illustrated below in **Figure 1.17**:

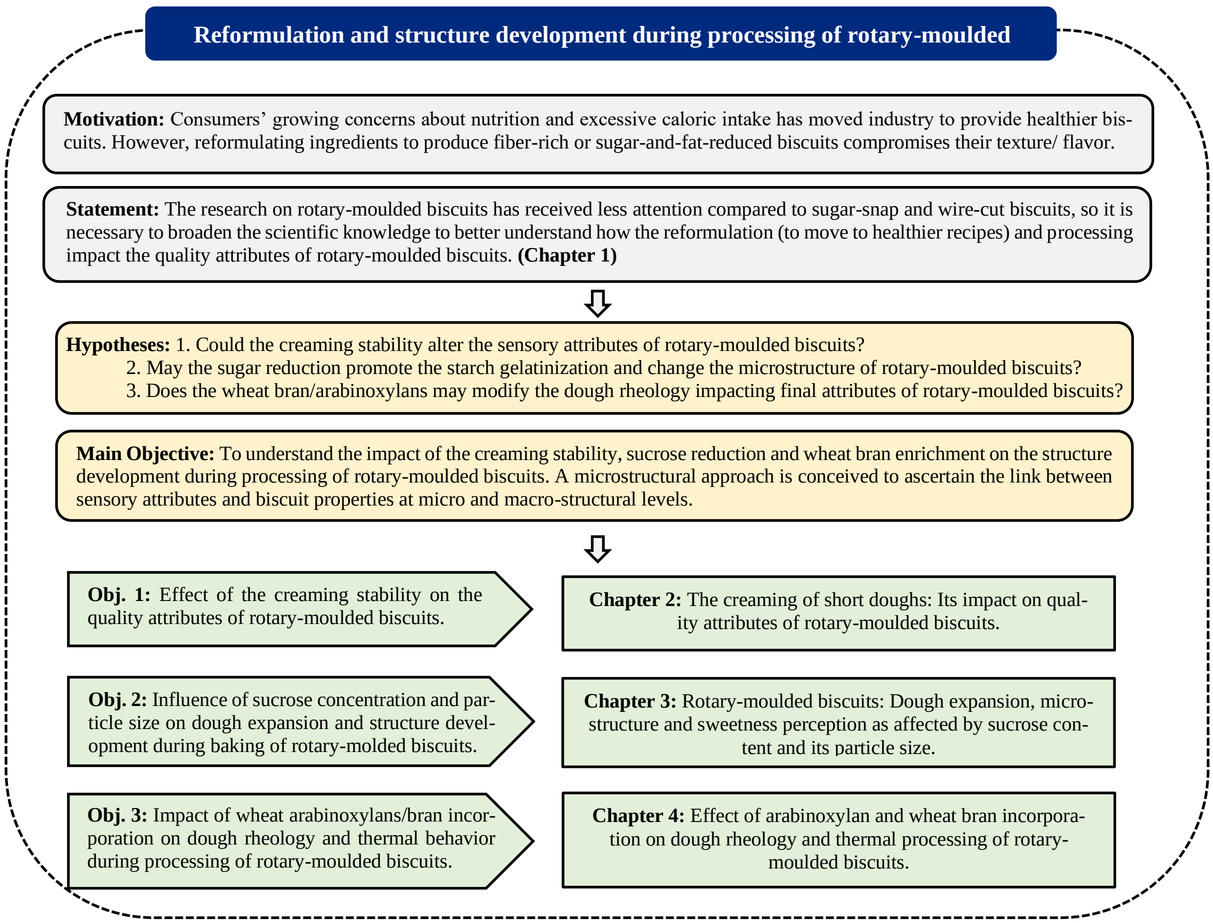


**Figure 1.17** Schematic overview of the thesis.

# 2. CHAPTER II: The creaming of short doughs: Its impact on quality attributes of rotary-moulded biscuits

**M. Teresa Molina**, Sandra M. Vaz & Pedro Bouchon

Being submitted to *MPDI/Foods Journal*, 2020.

## 2.1 Introduction

Biscuits are classified according to their formulation and the way they are produced, giving rise to the following biscuit categories: deposited, wafer, laminated, sugar-snap, rotary-moulded and wire-cut (Manley, 2011). Sugar-snap, rotary-moulded and wire-cut biscuits are obtained from a short dough, which differentiate them from the other classes as they have a higher amount of sugar and fat, and a lower amount of water (Kweon et al., 2014). In general, short doughs contain soft flour (9-11% total weight of protein), 17-33% of sugar (dough weight, w.b.) and 9-21% of fat (dough weight, w.b.) (Manley, 2011). However, there is a group of rotary-moulded dough that has lower amounts of sugar (10-20% dough weight, w.b.) and fat (6-12% dough weight, w.b.), and their effect on biscuit making has been less attended.

The mixing process is a critical stage on biscuit manufacture as it influences gluten development, and hence the rheological properties of the short dough (Manohar & Rao, 1999). Accordingly, horizontal mixers with a low rotation speed are usually used during this stage. The geometry of mixers may include single or double sigma blades, or a shaftless mixer blade. Industrial mixers operate between 30 and 70 rpm, whereas the speed of laboratory mixers may achieve up to 200 rpm (Davidson, 2018). The mixing process is generally divided into two steps in order to constrain the water-flour interaction as much as possible so that the gluten network formation is restricted, which are referred as the creaming (or cream-up) and the dough-up stages. During creaming preparation, fat, sucrose, water, leavening agents, and surfactant are blended together to produce a homogeneous, emulsified and aerated phase. During the dough-up step, the flour and the rest of the leavening agents are added to the creaming phase, at a low mixing speed and in the shortest possible time, to restrict the mechanical work and hindrance gluten development (van der Sman & Renzetti, 2018; Manley, 2011; Pareyt & Delcour, 2008a). At the end of mixing, a short dough is obtained, which is described as a suspension of proteins and starch granules in a continuous liquid sugar solution, were lipids are emulsified and constitute the disperse phase (Chevallier et al., 2000a). Sugars can be added to the dough formulation as crystals or as a sugar solution. Several studies have focused on the impact of sugar dissolution and its particle size on the quality attributes of sugar-snap and wire-cut biscuits, which have shown being crucial parameters during biscuit processing as they influence dough expansion during baking (Canalis et al., 2018; Laguna et al., 2013a; Pareyt et al., 2010b). It has been suggested that fine sucrose particles dissolve faster in water during baking, promoting the lateral spread of the dough that subsequently allows to obtain a larger and thinner biscuit compared to the effect of coarser crystals, which produce shorter and thicker biscuits as a consequence of their slower dissolution in water during heating (Kweon et al., 2009). Both, the sugar crystal size and the concentration of the sugar solution may provide a better understanding of their concomitant influence on the final attributes of rotary-moulded biscuits, which contain lower amounts of sugar than wire-cut or sugar snap biscuits, but as yet, no studies have addressed this effect in this biscuit category.

Furthermore, it has been stated that the creaming phase should be homogeneous and emulsified to prevent flour hydration during the dough-up phase (van der Sman & Renzetti, 2018; Kweon et al., 2014; Manley, 2011; Kamel, 1994). However, little attention has been given to understand whether an unstable creaming phase would actually have a negative effect on the final attributes of biscuits. An unstable creaming phase could potentially affect dough processing as water or the sugar solution may influence the functionality of flour polymers. Although the addition of a sugar solution delays the hydration of flour compared to water alone, this phenomenon is not inhibited (Slade & Levine, 1994). Kweon et al. (2011) declared that the hydration of gluten proteins with a sugar solution is thermodynamically more favorable than hydration with water alone, but the kinetics of gluten development is retarded when increasing the sugar concentration. Different mixing methods have been examined to assess the impact of flour hydration on the quality attributes of biscuits. Manohar & Rao (1999) observed that the elastic recovery and cohesiveness of rotary-moulded dough increased when all the ingredients were blended at once during mixing (all-in-one method), instead of when using two stages (with a creaming step), attributing this behavior to the gluten development due to higher water availability in the system. Regarding the mixing time and the mixing speed, most of the studies have focused on the effect of these parameters during the dough-up phase (Manohar & Rao, 1997; Contamine et al., 1995). However, it is still necessary to understand the impact of mixing parameters on the stability of the creaming phase, and how this condition may affect the quality attributes of biscuits. High-shear mixers offer higher rotation speeds than horizontal mixers, ranging from 1,000 to 25,000 rpm (Santana et al., 2013), but they are not commonly used during creaming elaboration. These types of mixers may provide a stable and emulsified creaming phase due to their high mechanical energy input, which increases the interfacial area of the disperse phase, and consequently may influence rotary-moulded dough performance.

Accordingly, the aim of this study was to determine whether an unstable or stable creaming phase may modify the quality attributes (i.e. dimensions, microstructure, hardness, and sensory profiling) of rotary-moulded biscuits when using different sucrose particle sizes and sugar concentrations when adding a sugar solution.

## 2.2 Materials and Methods

### 2.2.1 Rotary-moulded biscuits ingredients

The dough was prepared using commercial soft wheat flour (Molinera San Cristobal, Santiago, Chile) [composition: 70% starch, 13.9% moisture, 10% proteins, 3.6% total fiber, 2% lipids, 0.5% ash; 55% water absorption; 8.1% dry gluten; 4.3% damaged starch], palm oil (Teamfoods, Santiago, Chile), granulated or powdered sucrose (Iansa, Santiago, Chile), soy lecithin (Cargill, Santiago, Chile), ammonium bicarbonate (Basf, New Jersey, United States), sodium bicarbonate (Andimex S.A., Santiago, Chile), monocalcium phosphate (Blumos, Santiago, Chile), and salt (K+S Chile S.A., Santiago, Chile).

### 2.2.2 Biscuit formulation and preparation

The standard dough was formulated by mixing refined flour (65.2%, d.b.), sucrose (19.7%, d.b.), fat (12.3%, d.b.), leavening agents (2.5%, d.b.), soy lecithin (0.2%, d.b.), salt (0.1%, d.b.), and water (10.2% d.b.). Two sucrose particle sizes were used, granulated (GS, D90 = 978 µm) and powdered sucrose (PS, D90 = 98 µm). The amount of water (150 g, 10.2% d.b.) was divided into two fractions. Fifty g were used to dissolve the leavening agents and the salt in all formulations. The remaining portion (100 g) was used to prepare four sugar solutions, based on sugar saturation (ST), which are referred as 0%ST, 50%ST, 100%ST and >100%ST, in order to study the effect of the addition of a sugar solution on the stability of the creaming phase. These solutions were prepared considering the solubility of sucrose at 20°C (i.e. 204 g of sucrose per 100 g of water). 0%ST refers to pure water. In this case, 288 g of sucrose (19.7%, d.b.) and 100 g of water were separately added to the mix. The 50%ST solution means that 102 g of sugar were dissolved in 100 g of water, and the remaining fraction (186 g) was added as crystals to the formulation. The 100%ST solution means that 204 g of sugar were dissolved in 100 g of water, and the remaining 84 g were added as crystals to the mix. The >100%ST solution was an oversaturated sugar solution, which was obtained by dissolving 288 g of sucrose in 100 g of water, by heating and stirring at 70°C, to ensure the complete dissolution of sucrose crystals. This solution was finally cooled down and added to the dough preparation at 25 ± 1°C.

The dough was prepared in two steps, including a creaming and a dough phase. During the creaming step, the fat, the sugar crystals and/or the sugar solution, the sodium bicarbonate, the ammonium bicarbonate, the lecithin, the salt and the water were mixed during 5.5 min. This phase was prepared using two different mixers, in order to examine the effect of creaming stability on the quality attributes of rotary-moulded biscuits. To do so, either a horizontal mixer at the highest nominal speed (166 rpm average, Laboratory Z-blade mixer, Morton-Mixers, United Kingdom) or a vertical high-shear mixer at the highest nominal speed (9000 rpm, SL2T, Silverson, United Kingdom) were used. Thereafter, the flour and the monocalcium phosphate were added to the creaming phase and the mix was blended in a horizontal mixer for 60 s at minimum speed (85 rpm) to obtain the dough. The dough was moulded into 4×4 cm2 molds using a minilab rotary moulder (RTech Limited, United

Kingdom). Seventy-eight dough pieces per tray were baked at 150°C during 8 min using a convection oven (SALVA, Spain) until biscuits reached a final moisture content of 2.0 ± 0.2% (w.b.).

### 2.2.3 Aqueous-phase migration from the creaming phase

The stability of the creaming phase was analyzed by transferring the cream to a separating funnel, where it was left for phase separation until equilibrium (1 h at 21 ± 1°C). Thereafter, the aqueous phase was collected and weighted. The aqueous-phase migration was reported as the percentage of the aqueous-phase weight over the cream weight (Maskan & Göğüş, 2000).

### 2.2.4 Firmness of the creaming phase

The firmness of the creaming phase was determined at 21 ± 1°C using a penetration test in a TA.XT Texture Analyzer (5 kg loadcell, Stable Microsystems, United Kingdom), employing a conical TTC spreadability rig (HDP/SR). Before the analysis, the probe height was calibrated to ensure a starting point at the same height for each test (25.0 mm). The creaming phase was filled into the female cone, and the male cone was lowered up to 23 mm at a test speed of 3 mm/s. Five measurements per batch were taken. The force (N) at the maximum penetration depth was taken as a texture parameter of firmness (Glibowski et al., 2008).

### 2.2.5 Properties of rotary-moulded biscuits

The moisture content of biscuits was analyzed thermogravimetrically, using a HB43 halogen moisture analyzer (METTLER TOLEDO, USA), where 5 g of ground sample were heated at 125°C with a switch-off criterion 3. The dimensions of the biscuits (i.e. length, width and thickness) were measured with a digital vernier calliper (0-150 mm, Würth, Canada), and thirty biscuits per batch were evaluated. The maximum breaking force of biscuits was measured after two days, using a TA.XT Texture Analyzer (5 kg loadcell, Stable Microsystems, United Kingdom). Before the analysis, the samples were hermetically sealed using a trilaminate film PET/BOPP Met/PE, and they were stored at 20 ± 1°C. Twenty biscuits per batch were analyzed employing a three-point bending test (HDP/3PB probe) with a support span of 36 mm, using a test speed of 1 mm/s. The maximum breaking force (N) upon compression was used as a texture descriptor of biscuit hardness (Jambrec et al., 2013).

### 2.2.6 X-ray micro-computed tomography (X-ray μCT)

The microstructure of rotary-moulded biscuits was analyzed using a Skyscan 1272 X-ray micro-computed tomography system (version 1.1.7, Bruker Corp., Belgium), where the X-ray source operated at a voltage of 45 kV and a current of 222 μA. Images were acquired using an exposure time of 480ms, with a rotation step of 0.2° over an interval of 0-360°, and two frame averaging. Three samples were scanned for each condition.

Around 1800 projection images were obtained from the image acquisition, and they were further processed using reconstruction software NRecon v. 1.7.3 (Bruker Corp., Belgium) to obtain 2D cross-sectional images (resolution 2016 × 1344 pixels, voxel size of 10.7$\mu m^3$). During the reconstruction step, *thermal correction* (X/Y alignment with a reference scan), *misalignment compensation* (post-alignment), *smoothing* (1, using Gaussian Kernel = 2), *ring artifacts reduction* (= 5), and *beam-hardening correction* (= 45%) were set to obtain a good quality of the reconstructed images. The reconstructed images were processed and analyzed using CTAn software (version 1.17, Bruker Corp., Belgium), according to the following steps: (i) selection of a volume of interest (VOI); (ii) removal of residual noise; (iii) the definition of a region of interest (ROI) prior to segmentation; (iv) the segmentation of biscuit components (biscuit matrix and air pores); and (v) three-dimensional quantification using the structure thickness, which enables a 3D representation of the size distributions of biscuit components to be obtained (Bruker, 2019).

### 2.2.7 Sensory Analysis

The sensory attributes of biscuits (*i.e.* hardness, aeration level, noise intensity, grittiness, sweetness, color, and thickness) were analyzed with 10 trained panelists using the monadic profiling technique. The definition of each sensory attribute is presented in **Table 1**. The intensity of the sensory attributes was evaluated in two assessments, as recommended by Meyners et al. (2020) and Moser et al. (2018). An eleven-point hedonic scale was used during the evaluation, from 0 to 10, where the intensity ranges were defined as follows: 0.1 to 2 (weak); 2.1 to 4 (slightly weak); 4.1 to 6 (moderate); 6.1 to 8 (slightly strong); and 8.1 to 10 (strong). During the test, the panelists panelist inserted the sample into their mouth and the sensory attributes were evaluated during chewing of biscuit using FIZZ software (version 5.2, Biosystemes, France). After consuming each sample, panelists were instructed to rinse their mouth by drinking water and eating some plain crackers, as proposed by Kohyama et al. (2016), and to rest during 15 min before taking the following sample test. A total of five sessions were required to conduct the whole evaluation.

**Table 2.1** Definitions of sensory attributes analyzed by the trainee panel.

| Attribute | Definitions |
|---|---|
| Aeration | Visible air pores at the cross section of the biscuit. |
| Color | Level of roasted color at the surface of the biscuit. |
| Grittiness | Perception of sugar crystals between the tongue and palate or the tongue and teeth. |
| Hardness | Force required to break the biscuit after the first bite with incisors. |
| Noise intensity | Overall noise during chewing of biscuit. |
| Sweetness | Level of perceived sweetness during mastication of biscuit. |
| Thickness | How thick is the biscuit when looking at its cross-section. |

### 2.2.8 Statistical Analysis

The experimental data were acquired in triplicate (3 batches per condition) and they were analyzed with R (R Foundation for Statistical Computing, R Development Core Team), version 3.6.1. A one-way Welch ANOVA was applied when $n \geq 30$ (biscuit dimensions and maximum breaking force), in order to analyze whether the treatment means differed from each other. This test is used when homoscedasticity cannot be assumed but normally distributed data are required, where the central limit theorem approximation is good enough if the sample size is greater than 30 [26]. The pairwise post hoc analysis was performed using the Games–Howell test at 95% confidence, which does not assume homoscedasticity and equal sample sizes [27]. However, when n < 30, the bootstrap method, introduced by Efron [28], was used as it does not make any assumption on the underlying population distribution (i.e., a non-parametric approach) [29]. In particular, the analysis performed in this study did not assume that the data came from a normal distribution and instead it fully accepted that the population distribution was unknown. The bootstrap method uses sampling with replacement on the existing data to recover the unknown population distribution of the parameters of interest (mean in this case) and to estimate confidence intervals. To estimate this effect, one thousand replicates were created, and 95% confidence intervals were obtained to test differences between the means of the different formulations.

## 2.3 Results and Discussion

In order to determine whether the stability of the creaming phase has an impact on the quality and the sensory attributes of rotary-moulded biscuits, stable and unstable creams were prepared by modifying the mixer configuration, the sucrose particle size, as well as the concentration of the sucrose solution. Accordingly, the characterization of the creaming phase and the associated results are described in the following sections.

### 2.3.1 Characterization of the creaming phase

**Figure 2.1** shows the aqueous-phase migration, which was obtained as explained in section 2.2.3. It can be observed that when the cream was prepared using a low-shear (166 rpm) (**Figure 2.1.A and 2.1.B**), the sucrose particle size and the concentration of sugar in the sucrose solution affected the cream stability. Whereas granulated sucrose allowed to obtain a stable cream when adding either the 0%ST or the 50%ST solutions, the cream was unstable with powdered sucrose at all concentrations, and ~53-59% of the aqueous-phase migrated from the cream. During mixing of the creaming phase, two phenomena occur simultaneously: (i) sugar dissolution, and (ii) emulsifier adsorption at the interface between oil and water. Sugar dissolution depends on the sucrose particle size. Accordingly, powdered sucrose will probably dissolve faster than granulated sucrose because its surface-area-to-volume ratio is approximately four-times higher (28.1, 1/mm) than the value for granulated sucrose (6.7, 1/mm) (Molina et al., 2020). Moreover, in oil-in-water emulsions, stabilization of oil droplets requires mechanical energy to disrupt coarse droplets and an effective adsorption of the emulsifier, which is able to reduce the interfacial tension at the interface, strongly bind to the interface, and protect the oil droplets against flocculation and coalescence (Dickinson, 2009). Due to the slow rotational speed that may be achieved with the horizontal mixer, it is suggested that a stable creaming phase is difficult to obtain due to the insufficient disruptive force to fragment large oil droplets into smaller ones, as referred by McClements & Gumus (2016).

On the other hand, according to **Figures 2.1.C and 2.1.D**, it can be observed that the aqueous-phase migration was inhibited when using a high-shear mixer during creaming preparation, as neither the sucrose particle size nor the sugar solution affected the stability. The high-shear action of the mixer, and its disintegrating head geometry, most probably enhanced the homogenization of the water (or sugar solution) and the oil phase. The energy applied when using a high rotation speed (9000 rpm, nominal) help to increase the interfacial area of the dispersant phase (into droplets) and better distribute the surfactant (Tadros et al., 2004), so that a stable creaming phase may be obtained.

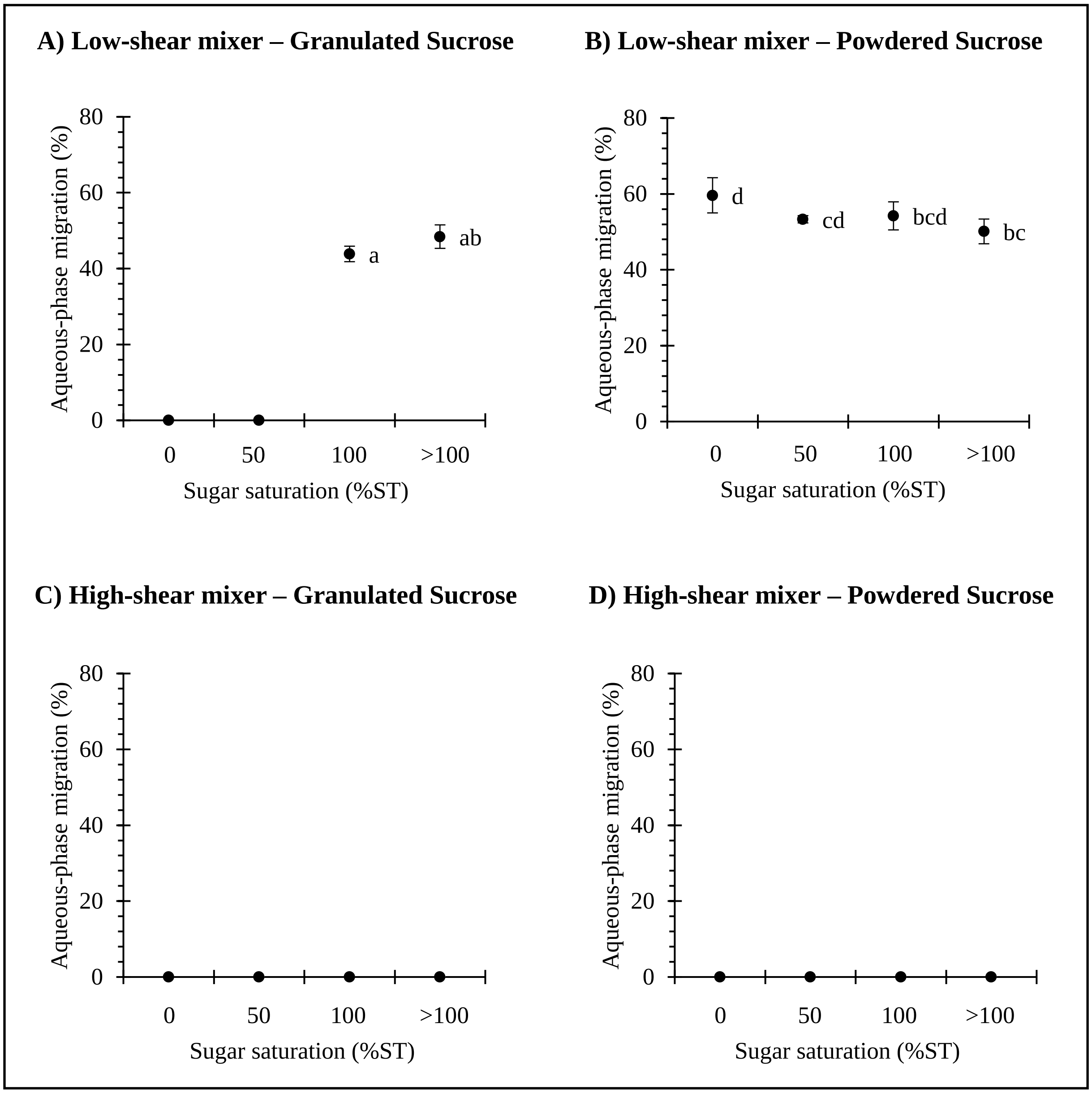


**Figure 2.1** Aqueous-phase migration (%) of the creaming phase, which was elaborated using granulated (A, C) or powdered (B, D) sucrose, and it was subjected to low-shear (A, B) or high-shear (C, D) mixing. Additionally, sucrose was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. Data are mean ± confidence intervals at 95% (n = 3). Different scripts denote significant differences (p-value < 0.05).

The firmness of the stable creaming phases was measured using a spreadability test, in order to understand whether this variable may influence the way biscuit dough spreads during baking and, consequently, whether it may be related to the final dimensions of biscuits. As shown in **Figure 2.2,** the highest firmness (~13 N) was obtained in creamings formulated with granulated sucrose and prepared using a low-shear mixer, and no significant differences (p-value > 0.05) were observed when water (0%ST) or an unsaturated sugar solution (50%ST) was used (**Figure 2.2.A**). The use of a high-shear mixer significantly decreased (p-value < 0.05) the firmness of the cream (**Figure 2.2.B and 2.2.C**), and the sucrose particle size affected this parameter. As a matter of fact, solutions prepared with powdered sucrose were consistently less firm than those prepared with granulate sucrose. The shearing action and the mixing time possibly are not enough to transform all the coarser sugar crystals (granulated sucrose) to finer particles with a similar distribution to that from powdered sucrose, leading to greater firmness. Interestingly, an increase in the amount of sugar dissolved in water tended to decrease the firmness of the cream. This could be related by the extra volume of the aqueous phase that is obtained as a result of sugar dissolution, as suggested by Ghiasi & RC (1983). However, when a >100%ST sugar solution was used, the firmness of the creaming phase incremented, and it was similar to the value observed in creams prepared with 0%ST sugar solution. This result did not follow the trend that was observed in the other samples, and it was expected to obtain a similar or even lower firmness value to that found in creams prepared with a sugar solution at 100% ST. This behavior could be related to sucrose crystallization induction in a supersaturated solution, which is catalyzed by the agitation energy input and the presence of small sugar crystals acting as secondary nuclei, as explained Hartel (2001). These factors could affect the sucrose-water interaction (Hartel et al., 2011) thus modifying the structure of the creaming phase to a system similar to that obtained at 0%ST sugar solution. Notwithstanding the foregoing, further studies are necessary to better understand the effect of sugar dissolution and saturation point on the rheology and the microstructure of the stabilized creaming phase of rotary-moulded biscuits.

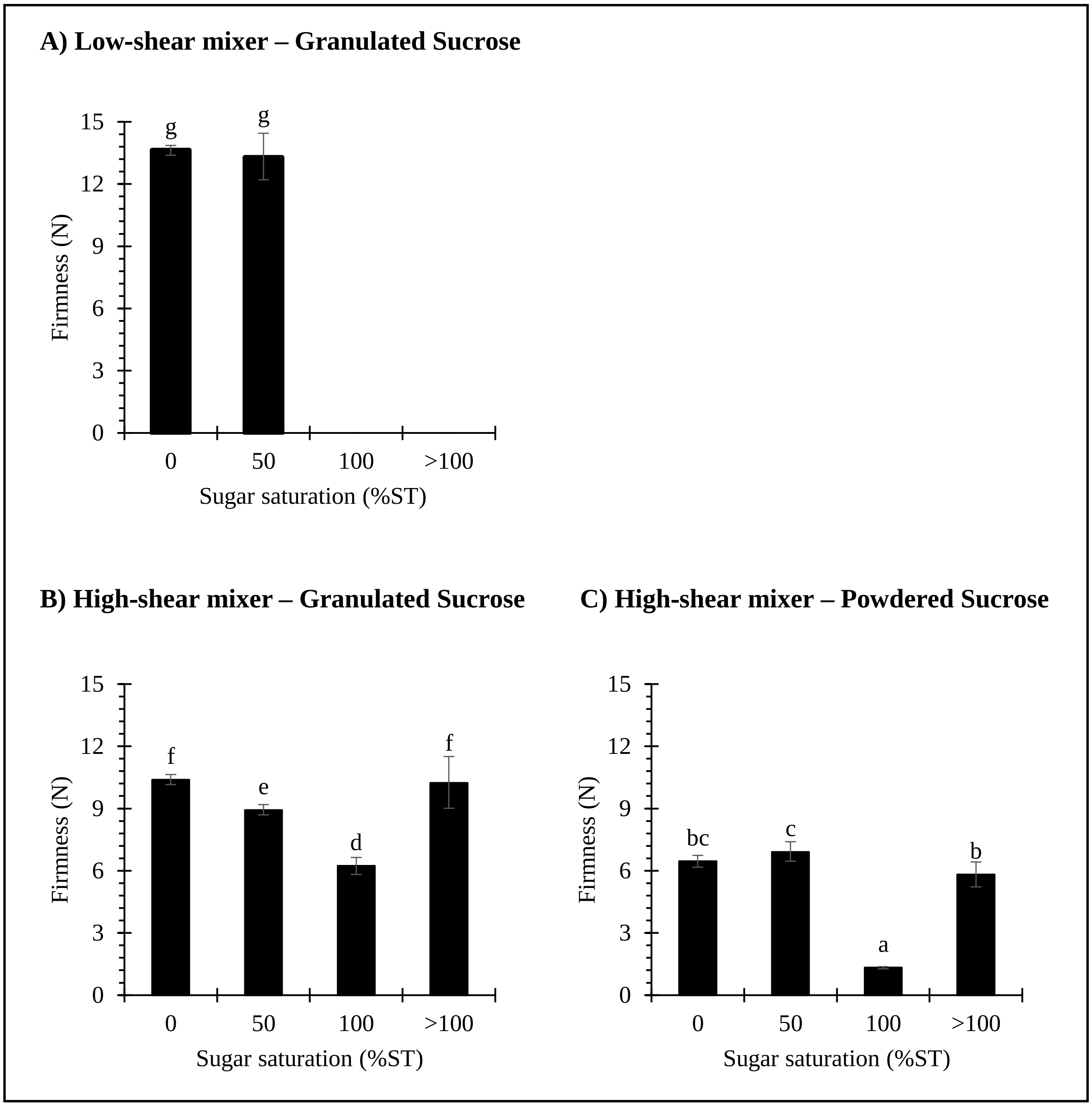


**Figure 2.2** Firmness (N) of the creaming phase, which was elaborated using granulated (A, B) or powdered (C) sucrose, and it was subjected to low-shear (A) or high-shear (B, C) mixing. Additionally, sucrose was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. Data are means ± confidence intervals at 95%. Different scripts denote significant differences ($p$-value $< 0.05$).

### 2.3.2 Dimensions of rotary-moulded biscuits

The dimensions of rotary-moulded biscuits were measured after baking. Results are reported in **Table 2.2**. Horizontal dimensions are linked to the lateral spread of dough during baking from its original size ($40 \times 40$ mm$^2$), while thickness is related to the dough vertical expansion. It can be observed that the length and the width were slightly smaller (p-value < 0.05) in biscuits whose creaming was prepared using the high-shear mixer compared to those subjected to low-shear mixing, with only one exception in both dimensions (granulated sucrose at >100%ST sugar solution). For instance, the length and width of samples elaborated with granulated sucrose and a sugar solution of 0%ST were 39.53 cm and 39.88 cm for biscuit prepared using a high-shear mixer, and 39.95 cm and 40.30 cm for that when a low-shear was used, respectively. Nevertheless, the differences did not exceed 0.8 mm in length (2%) and 0.25 mm in width (1%), so that the variation was considered rather negligible. These results may indicate that the differences observed in the firmness of the creaming did not modify the lateral spread of dough during baking because minor changes were obtained in the horizontal dimensions of rotary-moulded biscuits. In turn, the thickness was similar between samples, as no differences (p-value > 0.05) were found when comparing the effect of each mixer. When evaluating biscuits that were prepared using a low-shear mixing, it was determined that the sucrose particle size did not modify (p-value > 0.05) the length of biscuits, and the width was at the most 0.39 mm smaller (p-value < 0.05) in biscuits with powdered sucrose . A similar behavior was observed in biscuits prepared with high-shear mixing, where minor differences (< 0.45 mm) were detected among samples. Furthermore, the concentration of sucrose solution slightly affected biscuits dimensions, however, no clear pattern was observed, and the differences were not larger than 0.73 mm (1.8%) for the horizontal dimensions and 0.2 mm (3.7%) for the thickness. Previous studies have shown that the horizontal dimensions of sugar-snap and wire-cut biscuits are greater in products elaborated with finer sucrose crystals or when using a sugar solution (Kweon et al., 2009; Pareyt & Delcour, 2008a; Doescher & Hoseney, 1985). Particularly, Kweon et al. (2009) observed that biscuits prepared with coarser sugar crystals (> 500 µm) had smaller diameters than those prepared with finer crystals (< 500 µm), a result that was attributed to the slower dissolution of coarse crystals during mixing and baking. Overall, results presented in **Table 2.2** show that the sucrose particle size and the percentage of sugar dissolved in water practically do not modify the horizontal dimensions of rotary-moulded biscuits, as opposed to what has been found in wire-cut and sugar-snap biscuits.

**Table 2.2** Dimensions (*i.e.* length, width, thickness) of rotary-moulded biscuits, which were elaborated using granulated or powdered sucrose. Sucrose was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. The creaming phase was subjected to low-shear or high-shear mixing. Data of each dimension are means ± confidence intervals at 95%. Different scripts per dimension denote significant differences (p-value < 0.05).

| Type of mixer<br>Type of sucrose | Sugar saturation (%ST) | Creaming stability | Length (mm) | Width (mm) | Thickness (mm) |
|---|---|---|---|---|---|
| **Low-shear Granulated** | 0 | Stable | 39.95 ± 0.14 $^{cef}$ | 40.30 ± 0.06 $^{g}$ | 5.39 ± 0.04 $^{acde}$ |
| | 50 | Stable | 40.06 ± 0.17 $^{cde}$ | 40.27 ± 0.06 $^{gh}$ | 5.23 ± 0.05 $^{b}$ |
| | 100 | Unstable | 40.26 ± 0.12 $^{cd}$ | 40.35 ± 0.06 $^{g}$ | 5.43 ± 0.05 $^{cde}$ |
| | >100 | Unstable | 40.42 ± 0.16 $^{d}$ | 40.40 ± 0.07 $^{g}$ | 5.40 ± 0.05 $^{acde}$ |
| **High-shear Granulated** | 0 | Stable | 39.53 ± 0.12 $^{ab}$ | 39.88 ± 0.06 $^{abcd}$ | 5.29 ± 0.05 $^{ab}$ |
| | 50 | Stable | 39.38 ± 0.17 $^{a}$ | 39.88 ± 0.06 $^{acd}$ | 5.37 ± 0.04 $^{acde}$ |
| | 100 | Stable | 39.40 ± 0.15 $^{a}$ | 39.97 ± 0.07 $^{cde}$ | 5.45 ± 0.05 $^{cde}$ |
| | >100 | Stable | 40.11 ± 0.14 $^{cde}$ | 40.24 ± 0.10 $^{fgh}$ | 5.48 ± 0.06 $^{de}$ |
| **Low-shear Powdered** | 0 | Unstable | 40.26 ± 0.13 $^{cd}$ | 40.05 ± 0.06 $^{ef}$ | 5.35 ± 0.04 $^{acd}$ |
| | 50 | Unstable | 39.96 ± 0.36 $^{abcdef}$ | 40.04 ± 0.07 $^{cef}$ | 5.41 ± 0.06 $^{acde}$ |
| | 100 | Unstable | 39.96 ± 0.12 $^{ce}$ | 40.10 ± 0.08 $^{efh}$ | 5.40 ± 0.05 $^{acde}$ |
| | >100 | Unstable | 40.09 ± 0.14 $^{cde}$ | 40.01 ± 0.08 $^{ce}$ | 5.39 ± 0.06 $^{acde}$ |
| **High-shear Powdered** | 0 | Stable | 39.78 ± 0.15 $^{abef}$ | 39.76 ± 0.06 $^{ab}$ | 5.35 ± 0.04 $^{ac}$ |
| | 50 | Stable | 39.83 ± 0.16 $^{bef}$ | 39.79 ± 0.08 $^{abd}$ | 5.50 ± 0.06 $^{e}$ |
| | 100 | Stable | 39.50 ± 0.18 $^{ab}$ | 39.74 ± 0.08 $^{ab}$ | 5.39 ± 0.06 $^{acde}$ |
| | >100 | Stable | 39.66 ± 0.12 $^{abf}$ | 39.72 ± 0.07 $^{b}$ | 5.40 ± 0.05 $^{acde}$ |

### 2.3.3 Hardness of rotary-moulded biscuits

In order to understand the effect of creaming stability on the texture of rotary-moulded biscuits, the maximum breaking force was measured using a texture analyzer (**Figure 2.3**). The maximum breaking force in all biscuits ranged between 13.1 and 16.7 N. Moreover, biscuits whose creaming was prepared using a low-shear mixer (**Figures 2.3.A-2.3.B**) were between 1 and 3 Newton less hard (p-value < 0.05) than biscuits prepared with a high-shear mixer (**Figures 2.3.C-2.3.D**). For instance, in biscuits prepared with granulated sucrose and water (0%ST sugar solution), this value was ~14.2 N and 15.8 N in samples prepared using a low-shear (**Figure 2.3.A**) and a high-shear mixer (**Figure 2.3.C**), respectively. Furthermore, the concentration of sucrose solution did not impact (p-value > 0.05) the maximum breaking force except in biscuits prepared with granulated sucrose at >100%ST sugar solution. However, the difference was lower than 0.83 N (5.8%) and 1.13N (7.1%) compared to samples prepared with water (0%ST), when using low and high-shear mixing, respectively (**Figure 2.3.A** and **Figure 2.3.C**). In addition, the sugar crystal size did not influence the maximum breaking force (p-value > 0.05) in biscuits prepared with low-shear mixing and 0%ST, 50%ST and 100%ST sugar solutions. In turn, the sucrose particle size only influenced the maximum force at 50%ST in samples prepared with a high-shear mixer, where the value was only 1.65 N higher in biscuits prepared with granulated sucrose.

A sensory analysis was also performed to examine the perception of biscuit hardness by a trained panel. Additionally, the maximum breaking force and the hardness at the first bite were compared to ascertain whether the force increment within the range of 1 to 3 N could actually be perceived by the panelists. As can be seen in **Figure 2.4**, all biscuits were scored between 4.6 to 5.1, which corresponds to a moderate hardness in the intensity range. The concentration of sugar solution did not affect the hardness at the first bite except in biscuits prepared with powdered sucrose using a low-shear mixing at 50%ST and >100%ST sugar solution (**Figure 2.4.B**). Despite this difference, the results were in the same range within the sensory scale, and a variation of 0.27 between them may be considered negligible. Additionally, the differences determined with the texture analyzer (**Figure 2.3**) were not perceived by the sensory panelists. Regarding the sugar crystal size, this variable did not impact the hardness at the first bite (p-value > 0.05) (**Figure 2.4**), and the differences obtained instrumentally were not detected by the panelists. Furthermore, they did not detect any variation in biscuit hardness when its corresponding creaming phase was prepared using a low-shear or a high-shear mixer (**Figures 2.4.A-2.4.C** and **Figures 2.4.B-2.4.D**). Kim et al. (2012) prepared biscuits with different amounts of sugar to investigate the correlation between the hardness response obtained by three instrumental tests and by a trained sensory panel. When replicates of biscuits with the same amount of sugar were analyzed (for instance, 13.4% w.b.), even though the same level of hardness was detected by the panelists the bending test showed differences in hardness up to 4.6 N between samples. Similar results were obtained by Yılmaz & Öğütcü (2015), who also found that a difference of 5 N (measured using a texture analyzer) between two samples was not detected by the trained panel. Accordingly, it seems reasonable that a difference of 3 N measured instrumentally would not be perceived by trained panelists and even less by consumers.

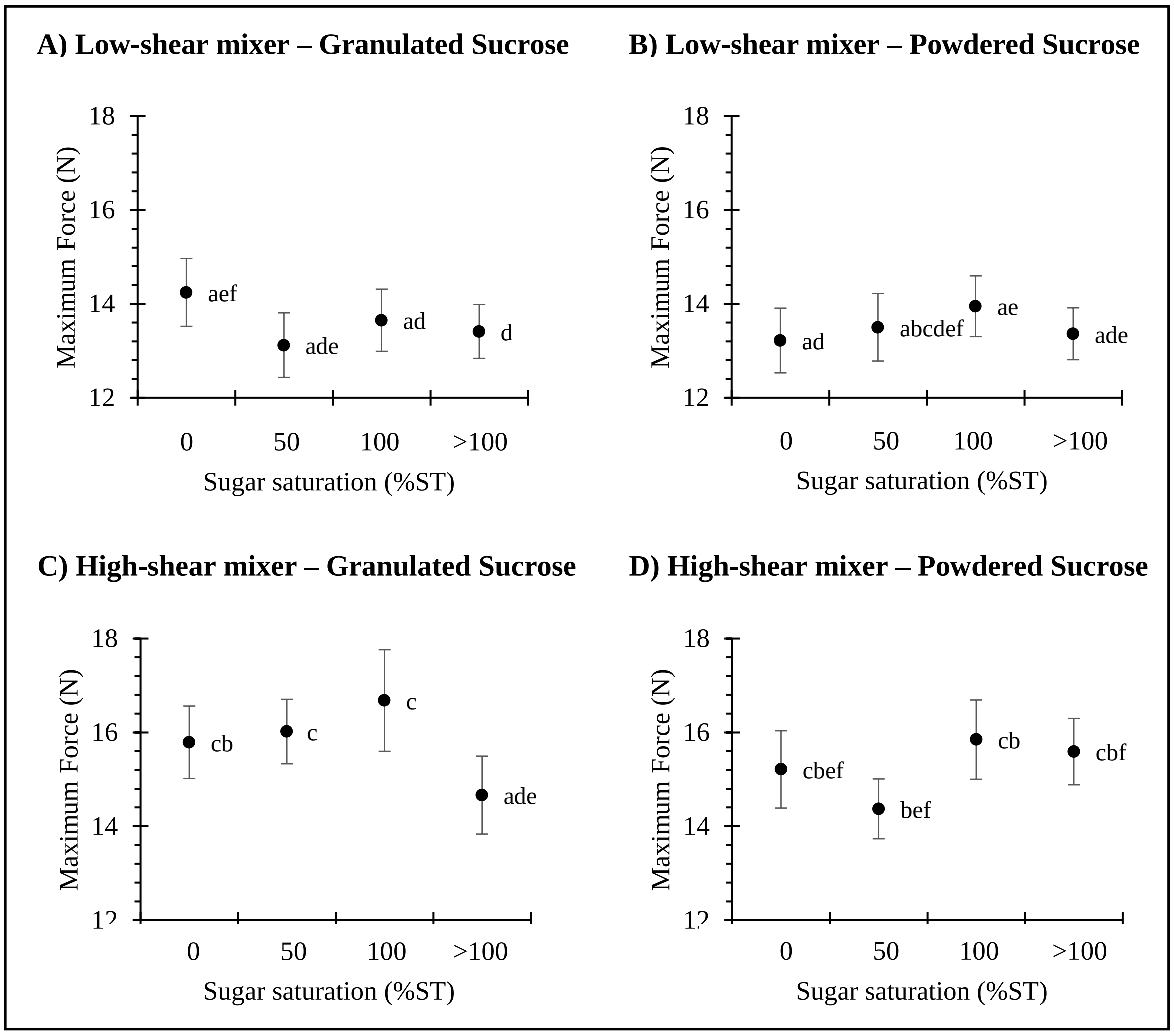


**Figure 2.3** Maximum breaking force (N) of rotary-moulded biscuits, obtained by texture analyzer. Biscuits were elaborated using granulated (A, C) or powdered (B, D) sucrose. Sugar was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. Also, the creaming phase was subjected to low-shear (A, B) or high-shear (C, D) mixing. Data are means ± confidence intervals at 95%. Different scripts denote significant differences (p-value < 0.05).

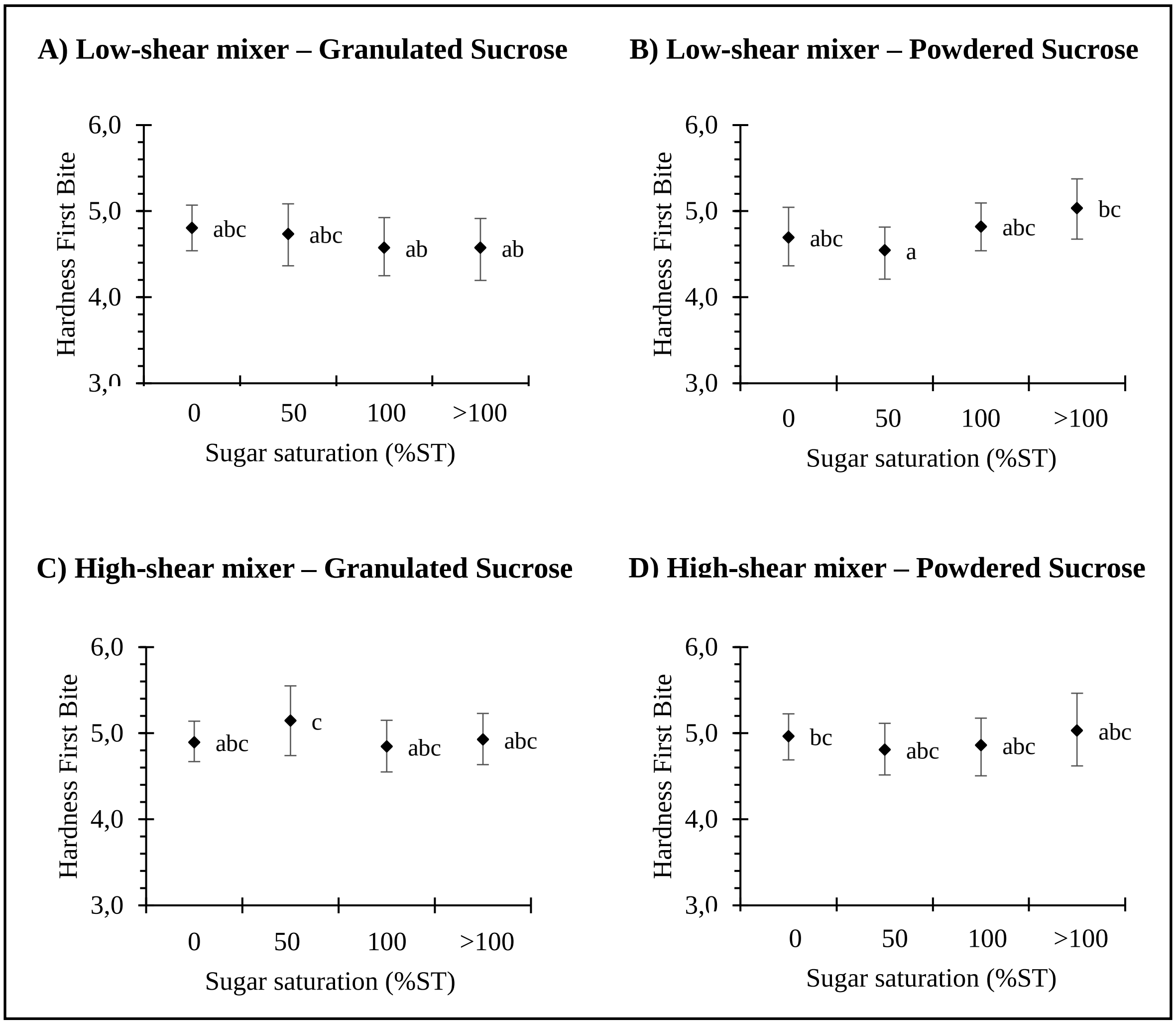


**Figure 2.4** Hardness at the first bite of rotary-moulded biscuits, obtained by a trainee sensory panel. Biscuits were elaborated using granulated (A, C) or powdered (B, D) sucrose. Sugar was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. Also, the creaming phase was subjected to low-shear (A, B) or high-shear (C, D) mixing. Data are means ± confidence intervals at 95%. Different scripts denote significant differences (p-value < 0.05).

### 2.3.4 Additional sensory attributes of rotary-moulded biscuits

To better understand the effect of creaming stability and sucrose (particle size, sugar in solution) on the sensory profiling of rotary-moulded biscuits, the aeration, the noise intensity, the grittiness, the sweetness, the color and the thickness were determined using a monadic profile with a trained sensory panel. **Figure 2.5** displays a two-dimensional radar graph, where the sensory attributes of different biscuits were compared. In addition, **Table 2.3** shows the statistical analysis and confidence intervals at 95% of these sensory attributes. It can be observed that all samples had similar sensory profiling (**Figure 2.5**), where the mean score range for each attribute was as follows: 2.7 to 3.3 for aeration, which corresponds to a slightly weak intensity; 4.4 to 4.9 for noise intensity, which is a moderate intensity; 2.0 to 2.5 for grittiness (slightly weak intensity), which is associated with the presence of sugar crystals, indicating that panelists did not perceive them; 5.0 to 5.4 for sweetness, which corresponds to a moderate intensity; 2.0 to 2.6 for color (slightly weak intensity), which is related to a light yellowish color; and 3.9 to 4.3 for thickness. Regarding the sucrose particle size, no significant differences (p-value > 0.05) were observed in none of the sensory attributes when biscuits were prepared with a low-shear mixer (**Table 2.3**). The same trend was observed in samples prepared using a high-shear mixer, apart from color, where a slight but minor difference was observed between samples prepared with >100%ST sugar solution. Furthermore, at equal sugar crystal size, the type of mixer did not influence the sensory attributes (p-value > 0.05) at any concentration of sugar solution. Accordingly, these results also show that a stable or unstable creaming phase does not produce any variation on the sensory attributes of rotary-moulded biscuits.

Regarding the concentration of sucrose solution used for creaming preparation, only in four attributes some significant differences between samples (p-value < 0.05) were found (bold marks in **Table 2.3**): aeration in samples "B" with 50%ST and >100%ST sugar solutions; sweetness in samples "A" with 50%ST and 100%ST sugar solutions; color in samples "D" with 50%ST and >100%ST sugar solutions; and thickness in samples "C" with 0%ST and >100%ST sugar solutions. Despite this, the intensity range was similar between each pair, and no specific relationship was found among these samples. Furthermore, the sweetness and the grittiness were similar between samples, indicating that these attributes were not perceived different when sucrose was incorporated as crystal or pre-dissolved in water. Analyzing the biscuit formulation, the sucrose/added water ratio was 1.9 $\frac{g\ sucrose}{g\ water}$. In most of the samples, part of sugar crystals was pre-dissolved before mixing, but also a fraction remained as crystals. Bearing in mind that the solubility of sucrose increases with temperature, for instance from 2.07 $\frac{g\ sucrose}{g\ water}$ at 25°C to 4.76 at 100°C (Bubník & Kadlec, 1995), it is suggested that the remaining part of sugar crystals can be completely dissolved during baking when the sucrose/added water ratio is much below the solubility of sucrose at 100°C.

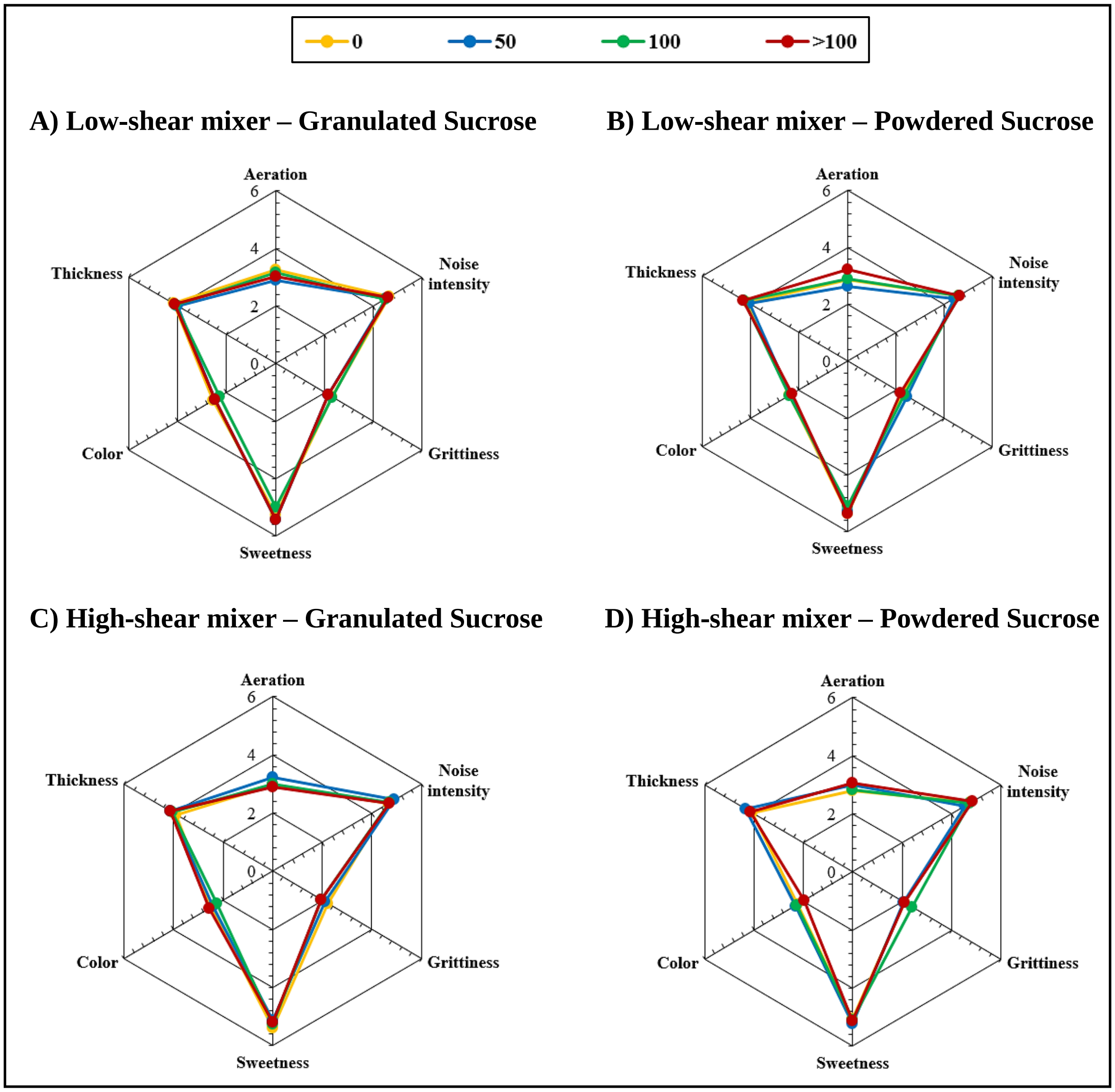


**Figure 2.5** Sensory attributes (*i.e.* aeration, noise intensity, grittiness, sweetness, color, thickness) of rotary-moulded biscuits. Biscuits were elaborated using granulated (A, C) or powdered (B, D) sucrose. Sugar was dissolved in water to obtain the following sugar solutions: 0%ST (yellow), 50%ST (blue), 100%ST (green) or >100%ST (red). Also, the creaming phase was subjected to low-shear (A, B) or high-shear (C, D) mixing. Data (n = 20) are expressed as observed mean.

**Table 2.3** Confidence intervals at 95% of sensory attributes for rotary-moulded biscuits. Biscuits were elaborated using granulated or powdered sucrose. Sugar was dissolved in water to obtain the following sugar solutions: 0%ST, 50%ST, 100%ST or >100%ST. Also, the creaming phase was subjected to low-shear (A, B) or high-shear (C, D) mixing. Different scripts per attribute denote significant differences (p-value < 0.05).

| **Sensory Attributes** | **Aeration** | **Noise** | **Grittiness** | **Sweetness** | **Color** | **Thickness** |
|---|---|---|---|---|---|---|
| **Sugar saturation (%ST)** | **A: Low-shear mixer – Granulated sucrose** | | | | | |
| 0 | (2.94, 3.63) $^{b}$ | (4.31, 5.01) $^{a}$ | (1.88, 2.68) $^{a}$ | (5.00, 5.56) $^{ab}$ | (2.20, 2.92) $^{b}$ | (3.98, 4.43) $^{abc}$ |
| 50 | (2.64, 3.14) $^{ab}$ | (4.07, 4.92) $^{a}$ | (1.72, 2.55) $^{a}$ | **(5.19, 5.65) $^{b}$** | (2.17, 2.74) $^{b}$ | (3.86, 4.16) $^{ab}$ |
| 100 | (2.94, 3.45) $^{b}$ | (4.10, 4.88) $^{a}$ | (1.91, 2.77) $^{a}$ | **(4.73, 5.29) $^{a}$** | (2.02, 2.54) $^{ab}$ | (3.96, 4.19) $^{abc}$ |
| >100 | (2.68, 3.37) $^{ab}$ | (4.23, 5.02) $^{a}$ | (1.69, 2.59) $^{a}$ | (5.08, 5.73) $^{ab}$ | (2.21, 2.70) $^{b}$ | (3.99, 4.25) $^{abc}$ |
| | **B: Low-shear mixer – Powdered sucrose** | | | | | |
| 0 | (2.55, 3.16) $^{ab}$ | (4.19, 5.01) $^{a}$ | (1.82, 2.82) $^{a}$ | (5.01, 5.70) $^{ab}$ | (2.18, 2.60) $^{b}$ | (4.00, 4.26) $^{bc}$ |
| 50 | **(2.32, 2.94) $^{a}$** | (4.03, 4.77) $^{a}$ | (2.06, 2.85) $^{a}$ | (4.96, 5.57) $^{ab}$ | (2.09, 2.65) $^{ab}$ | (3.80, 4.33) $^{abc}$ |
| 100 | (2.61, 3.18) $^{ab}$ | (4.22, 5.00) $^{a}$ | (1.89, 2.81) $^{a}$ | (4.74, 5.43) $^{ab}$ | (2.10, 2.69) $^{b}$ | (4.07, 4.55) $^{c}$ |
| >100 | **(2.97, 3.56) $^{b}$** | (4.30, 5.02) $^{a}$ | (1.86, 2.59) $^{a}$ | (5.04, 5.65) $^{ab}$ | (2.03, 2.51) $^{ab}$ | (4.01, 4.66) $^{bc}$ |
| | **C: High-shear mixer – Granulated sucrose** | | | | | |
| 0 | (2.73, 3.24) $^{ab}$ | (4.24, 5.09) $^{a}$ | (1.87, 2.57) $^{a}$ | (5.09, 5.67) $^{ab}$ | (2.29, 2.67) $^{b}$ | **(3.70, 4.06) $^{a}$** |
| 50 | (2.90, 3.61) $^{b}$ | (4.54, 5.34) $^{a}$ | (1.72, 2.48) $^{a}$ | (4.87, 5.40) $^{ab}$ | (2.13, 2.68) $^{b}$ | (3.95, 4.28) $^{abc}$ |
| 100 | (2.65, 3.39) $^{ab}$ | (4.38, 5.11) $^{a}$ | (1.62, 2.25) $^{a}$ | (4.99, 5.55) $^{ab}$ | (2.02, 2.50) $^{ab}$ | (3.88, 4.24) $^{abc}$ |
| >100 | (2.62, 3.18) $^{ab}$ | (4.29, 5.16) $^{a}$ | (1.62, 2.30) $^{a}$ | (4.89, 5.54) $^{ab}$ | (2.29, 2.80) $^{b}$ | **(3.97, 4.34) $^{bc}$** |
| | **D: High-shear mixer – Powdered sucrose** | | | | | |
| 0 | (2.44, 3.20) $^{ab}$ | (4.44, 5.15) $^{a}$ | (1.67, 2.52) $^{a}$ | (4.81, 5.28) $^{a}$ | (1.92, 2.49) $^{ab}$ | (3.78, 4.36) $^{abc}$ |
| 50 | (2.67, 3.33) $^{ab}$ | (4.17, 4.95) $^{a}$ | (1.70, 2.46) $^{a}$ | (4.94, 5.51) $^{ab}$ | **(2.17, 2.49) $^{b}$** | (4.09, 4.56) $^{c}$ |
| 100 | (2.46, 3.25) $^{ab}$ | (4.33, 5.19) $^{a}$ | (1.95, 2.81) $^{a}$ | (4.83, 5.36) $^{ab}$ | (2.02, 2.50) $^{ab}$ | (3.83, 4.42) $^{abc}$ |
| >100 | (2.84, 3.32) $^{ab}$ | (4.51, 5.22) $^{a}$ | (1.68, 2.45) $^{a}$ | (4.84, 5.41) $^{ab}$ | **(1.68, 2.25) $^{a}$** | (3.97, 4.36) $^{abc}$ |

### 2.3.5 Microstructure of rotary-moulded biscuits

The mechanical properties of biscuits are related to their microstructure and affect the perception of their sensory attributes during chewing (Baltsavias et al., 1997). To better understand the effect of the creaming stability on the microstructure of biscuits, the air porosity and the size distribution of air pores was analyzed (3D quantification), as shown in **Figure 2.6**. Only biscuits prepared with water (0%ST sugar solution) were selected as previous results showed that the concentration of sugar solution did not alter neither the maximum breaking force nor the sensory attributes of biscuits. **Figure 2.6.A** presents the 2D cross-sectional images of biscuits prepared with an unstable or stable creaming phase, where the air pores are represented in black and the biscuit matrix in beige white. The air porosity (%) was measured from 3D micro-CT images and corresponded to the percentage of the volume of voxels of air pores over the total volume of voxels. All samples had a similar air porosity (p-value > 0.05), which is consistent with that obtained in the aeration attribute by sensory analysis, and their mean values were 47.6% (**Figure 2.6.A.i**), 47.8% (**Figure 2.6.A.ii**), 47.0% (**Figure 2.6.A.iii**) and 47.2% (**Figure 2.6.A.iv**), respectively. Besides this information, the structure thickness distribution of air pores was also examined through micro-CT images analysis, as shown in **Figure 2.6.B**. Around 30% of air pores were smaller than 96 µm, ~70% of them were lesser than 225 µm, and about 98% were located below 546 µm, which is in agreement with Pareyt et al. (2009a), who found that a sugar-snap biscuit with 17.6% sugar (w.b.) had most of its air pores below 637.26 µm. Moreover, at equal sucrose particle size, the type of mixer did not produce significant differences (p-value > 0.05) in the air pores size distribution of rotary-moulded biscuits, except for biscuits with powdered sucrose in the range of [96, 225) µm. The difference was not pronounced because biscuit prepared with high-shear mixer (stable creaming) had 42.6% of air pores located in that range compared to 40.2% for biscuit prepared with low-shear mixer (unstable creaming).

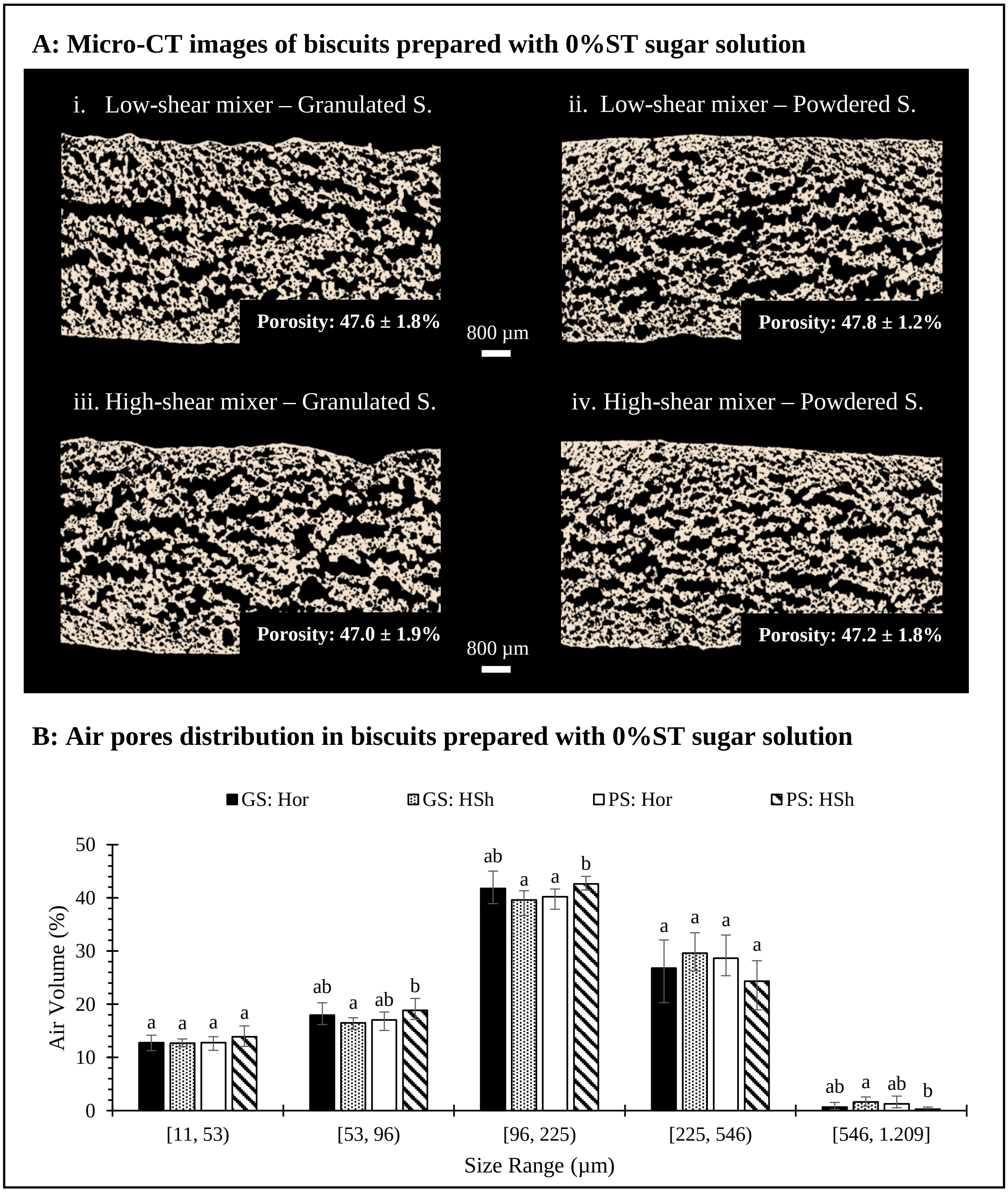


**Figure 2.6** 2D X-Y cross-sectional images (by X-ray µCT) of biscuits (A) and structure-thickness distribution of air pores inside the biscuit structure (B), which were formulated with sugar solution at 0%ST, using granulated (GS) or powdered (PS) sucrose, and the creaming phase was subjected to low-shear (Hor) or high-shear (HSh) mixing. Porosity is means ± standard deviation (n = 3). Data from air pores distribution are means ± confidence intervals at 95%. Different scripts in porosity or per range interval in air pores distribution denote significant differences ($p < 0.05$).

Furthermore, the wall thickness distribution of the biscuit matrix was quantified (3D quantification) also through micro-CT image analysis, as shown in **Figure 2.7**. It can be observed that the thickness of biscuit walls is within the range of 21 and 200 µm, and no differences were obtained among the cumulative distributions of samples. For instance, the $D_{50}$ and $D_{80}$ were 86.4 ± 0.1 and 108.8 ± 0.2 for biscuit formulated with granulated sugar and a stable creaming phase (**Figure 2.7.A**), 84.0 ± 1.0 and 105.6 ± 0.6 for biscuit elaborated with powdered sugar and unstable creaming phase (**Figure 2.7.B**), 86.2 ± 0.7 and 108.6 ± 1.5 for biscuit elaborated with granulated sugar and a stable creaming phase by high-shearing (**Figure 2.7.C**), and 82.5 ± 0.3 and 104.5 ± 0.2 for biscuit prepared with powdered sugar and a stable creaming phase by high-shearing (**Figure 2.7.D**). These results show that the stability of the creaming phase did not modify the thickness distribution of inner walls in rotary-moulded biscuits, which could explain the similar results obtained when analyzing the texture and the remaining sensory attributes.

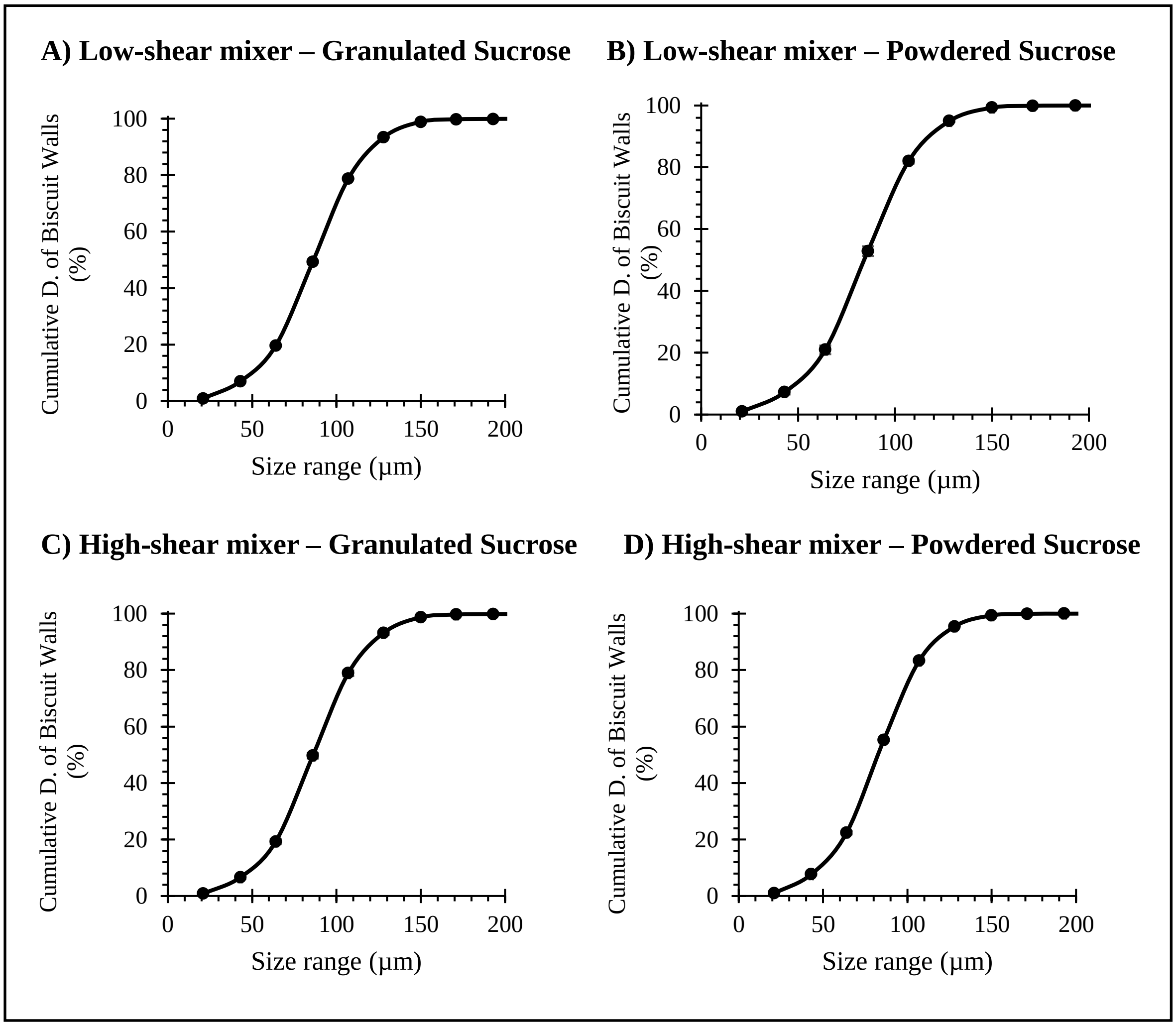


**Figure 2.7** Structure thickness cumulative distribution of the biscuit's walls, which were elaborated with sugar solution at 0%ST, using granulated (A, C) or powdered (B, D) sucrose, and the creaming phase was subjected to low-shear (A, B) or high-shear (C, D) mixing. Data are means ± standard deviation (n = 3).

## 2.4 Conclusions

This study examined the effect of creaming stability on the quality attributes of rotary-moulded biscuits. Results showed that creaming was unstable when it was produced using in a low-shear mixer, where the sucrose particle size and saturated or supersaturated sugar solution also influenced the aqueous-phase migration. However, the use of a high-shear allowed to obtain a stable creaming phase. Despite the variation on creaming stability, no significant differences were observed in the sensory profile (aeration, noise intensity, grittiness, sweetness, and color) of rotary-moulded biscuits. In addition, the variation of biscuit dimensions was either inexistent or negligible, according to micro-CT analysis, which revealed that biscuits prepared with an unstable or a stable creaming phase had a similar air porosity and thickness of biscuit walls. Until now it was generally accepted that the creaming phase of short doughs had to be stable and emulsified in order to obtain an adequate biscuit. This study suggests that the stability of the creaming phase does not seem to be a relevant factor to determine the quality attributes of rotary-moulded biscuits, using the formulations and conditions studied herein.

## 2.5 Acknowledgements

The authors are thankful to Katherine Allel and Michela Lauriola for their technical assistance in sensory analysis. Furthermore, the authors would like to acknowledge the financial support of CONICYT through the Advanced National Human Capital Formation Program - Doctoral Fellowship 2016 (Folio n° 21161243), and the NESTLÉ-UC Research Agreement.

# 3. CHAPTER III: Rotary-moulded biscuits: Microstructure development and dough expansion affected by sucrose concentration and its particle size

**M. Teresa Molina**, Sandra M. Vaz, Ángel Leiva & Pedro Bouchon

Submitted to *Journal of Food Structure*, 2020.

## 3.1 Introduction

Sucrose is the most important sugar used in biscuit manufacturing. However, high sugar consumption is associated with an escalating caloric intake and as a factor that causes obesity and dental decay (Pareyt et al., 2009a). These concerns are driving governments to implement front-of-package (FoP) nutrition labeling policies to enable consumers to make better informed choices (EUFIC, 2018). In the biscuit industry, there is a need to reduce the amount of added sugar in order to produce biscuits without FoP nutrition labels (van der Sman & Renzetti, 2018).

Research on biscuit formulation and manufacturing has been mainly focused on sugar-snap and wire-cut biscuits, likely due to their popularity (Canalis et al., 2018; Laguna et al., 2013a; Kweon et al., 2009; Pareyt et al., 2009b; Slade & Levine, 1994). The levels of sugar and fat in these biscuits are in the range of between 17-33% and 9-21% of the total dough weight (w.b.), respectively (Manley, 2011). Rotary-moulded is another biscuit category, which may be produced with lower amounts of sugar (10-20% w.b.) and fat (6-12% w.b.). The accumulated knowledge for this group of rotary-moulded biscuits has been limited; consequently, current scientific knowledge cannot be directly applied to this specific biscuit category.

The low water content in sugar-snap and wire-cut doughs, together with the high sugar and fat concentrations, delays the aggregation of gluten proteins and precludes starch gelatinization during baking (Kweon et al., 2014), which enables biscuit dough to expand for a longer period of time during baking. Accordingly, Pareyt et al. (2009b) concluded that gluten proteins in sugar-snap biscuits are not functionally inert during baking, while also observing that protein aggregation is partly inhibited in doughs with a high sugar content (39.5% w.b.), thus extending the time in which the expansion of dough occurs. However, there is still no consensus about whether starch gelatinization takes place during biscuit baking, and thus whether it is responsible for limiting dough expansion. While Laguna et al. (2013a) and Chevallier et al. (2000a) showed that starch granules partially gelatinized (< 50%) in doughs with 21% to 26% (d.b.) of sugar content, Pareyt et al. (2009a) and Pareyt & Delcour (2008a) concluded that no gelatinization occurred in doughs with 25% to 35% (d.b.) of sugar content, explaining that starch granules primarily act as filler components to support the biscuit matrix. Therefore, further data is needed.

Slade & Levine (1994) found that in dough with a high concentration of sucrose, the rate of sucrose dissolution depends on its particle size, influencing the way dough expands during baking. They explained that sugars act as antiplasticizers when they are dissolved in water, thus a smaller sucrose particle size (baker's special vs fine granulated) may delay or even prevent the thermal transitions of starch and gluten proteins, promoting the spreading and expansion of the dough. Accordingly, Kweon et al. (2009) formulated wire-cut and sugar-snap biscuit dough using sucrose with two particle sizes, ultra-fine (particle size lower than 500 µm) or fine-granulated (particle size higher than 500 µm), and showed that coarser sucrose crystals retard their dissolution in water, suggesting that this behavior may promote gluten development and starch gelatinization. As a consequence, biscuits with a smaller diameter were obtained as compared to those made with smaller sucrose crystals. Besides the effect of sugar particle size on baking performance, the perception of sugar crystals during the oral processing of biscuits has been linked to important sensory attributes, such as grittiness/crunchiness and noise intensity (van der Sman & Renzetti, 2018; Laguna et al., 2013b; Manley, 2011). However, no studies have been found referring to the effect of sucrose particle size on the sweetness perception of biscuits, a key aspect when focusing on the reduction of sucrose content.

The impact of dough spreading on sugar-snap or wire-cut biscuits has been usually characterized by analyzing their final dimensions and the spread factor (length-to-width ratio). This is one of the main quality indicators as it reflects the magnitude of lateral spread and vertical expansion during baking (van der Sman & Renzetti, 2018; Kweon et al., 2014). The inner structure of biscuits has been observed using scanning electron microscopy, which allows 3D visualization of different components, including fat, starch granules and the gluten structure (Mamat & Hill, 2014; Rodríguez-García et al., 2013; Pareyt et al., 2010a). However, it is difficult to derive quantitative information from SEM images. X-ray micro-computed tomography (X-ray µCT) is a non-invasive microscopy technique that allows 3D quantification (~ 1µm resolution) of the specimen inner structure, and it has been increasingly used in food analysis (Contardo & Bouchon, 2018; Schoeman et al., 2016). However, few studies have been developed to analyze the biscuit microstructure (Yang et al., 2012; Frisullo et al., 2010; Pareyt et al., 2009a), and even fewer related to the effect of sugar addition (Pareyt et al., 2009a). These authors showed that sugar reduction in sugar-snap biscuits not only decreases the porosity of the biscuit, but also provides a more homogeneous distribution of air pore size. Therefore, an in-depth use of this technique may lead to a better understanding of the effect of product formulation and processing conditions on aeration, porosity distribution and the overall structural development of rotary-moulded biscuits.

Accordingly, the aim of this study was to analyze the influence of sucrose reduction and particle size on dough expansion and starch gelatinization during baking of rotary-moulded biscuits using a microstructural approach, and their link to sweetness perception during oral processing.

## 3.2 Materials and Methods

### 3.2.1 Rotary-moulded biscuit ingredients

The rotary-moulded biscuit dough was prepared using commercial soft wheat flour (Molinera San Cristóbal, Santiago, Chile) [composition: 70% starch, 13.8% moisture, 10% proteins, 3.6% total fiber, 2% lipids, 0.6% ash], palm oil (Teamfoods, Santiago, Chile), granulated or powdered sucrose (Iansa, Santiago, Chile), soy lecithin (Cargill, Santiago, Chile), leavening agents (sodium bicarbonate from Andimex, Chile; ammonium bicarbonate from

Basf, Chile; and monocalcium phosphate from Budenheim, USA), and salt (K+S Chile S.A., Santiago, Chile).

### 3.2.2 Biscuit formulation and preparation

The biscuit dough was made in triplicate (3 batches were prepared for each formulation). The standard dough was prepared as follows. First, the sucrose (36.2%, w.b.), fat (10.5%, w.b.), water (5.8%, w.b.), soy lecithin (0.3%, w.b.), salt (0.2% w.b.) and part of the leavening agents (1% w.b.) were mixed during 5.5 min using a horizontal mixer (Morton Machines, United Kingdom) at maximum speed (~166 rpm), in order to reach the creaming phase. Following this, the flour (45%, w.b.) and the rest of the leavening agents (1%, w.b.) were then added to this phase. The mix was blended for 60 s at a minimum speed (~85 rpm). The dough was then moulded into 4×4 $cm^2$ molds using a minilab rotary moulder (RTech Limited, United Kingdom). Seventy-eight doughs per tray were baked at 150°C in a convection oven (SALVA, Spain) until the biscuits reached a final moisture content of 2.8 ± 0.3% (w.b.).

To analyze the effect of sugar reduction and sugar particle size during processing, the proportion of all ingredients, in a dry basis (d.b.), was maintained constant, except for the sugar content, which was progressively reduced whereas the flour content was increased. However, a constant sum of the amount of sugar and flour (d.b.) was ensured in all formulations. Accordingly, four sucrose concentrations were examined (d.b.): 40% (standard dough), 27%, 19% and 10%, leading to four sucrose:flour ratios, i.e.: 0.9, 0.5, 0.3 and 0.1, to which we will refer to further on. It was also possible to adjust the water content to enable dough molding, ensuring a similar water activity (0.85 ± 0.05) in all doughs. Two sucrose particle sizes were used, granulated sucrose (GS) and powdered sucrose (PS), the size distributions of which were characterized using laser light scattering.

### 3.2.3 Sugar particle size distribution ascertained by laser light scattering

The particle size distribution of GS and PS was measured in duplicate using a MasterSizer® 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd, UK), following a procedure adopted by Nestlé. Before the analysis, the sample was placed into an automatic dry dispersion unit (Aero S, Malvern Instruments Ltd, UK). The measurements were performed in accordance with the Mie theory, considering an absorption and refractive index for sucrose of 0.01 and 1.538, respectively. Data were collected using MasterSizer® 3000 software. After obtaining the particle size distribution, an approximation of the surface-area-to-volume ratio was calculated according to **Eq. (3.1)**, assuming a spherical shape of particles (Allen, 2013):

$$\text{Surface area to volume ratio} = \frac{particle\ surface\ area}{particle\ volume} = \frac{4 \cdot \pi \cdot \left(\frac{d_s}{2}\right)^2}{\frac{4}{3} \cdot \pi \cdot \left(\frac{d_v}{2}\right)^3} = \frac{6}{d_{sv}} \tag{3.1}$$

where $d_s$ is the diameter of a sphere that has the same surface area as the particle of interest (mm), $d_v$ is the diameter of a sphere that has the same volume as the particle of interest (mm), and $d_{sv}$ is the surface-volume mean diameter (mm), known as the Sauter diameter $D_{32}$, which is the diameter of a sphere that has the same ratio of surface to volume as the particle of interest.

### 3.2.4 Time-lapse photography

Time-lapse photography was performed according to Pareyt et al. (2008b), with some modifications. The vertical expansion of the biscuit dough during baking was recorded with a digital camera (Nikon D7200 18-140 VR, Thailand), with the purpose of quantifying the biscuit height as a function of baking time, every 30 s. Three biscuit doughs were followed per video recording, and three replicates were baked for each formulation. Images were processed using a sharpening filter (7 × 7 *Hipass*, 1 pass, and 5 strength; 5 × 5 *Sharpen*, 1 pass, and 5 strength) and analyzed using Image ProPlus 4.5 software (Media Cybernetics, USA). The vertical expansion (mm) was measured at the center of the biscuit, and it was expressed as the height difference between time t ($h_t$) and time zero ($h_0$), *i.e.*($h_{t=i} - h_{t=0}$), where $h_0$ was 3.5 mm.

### 3.2.5 Differential Scanning Calorimetry (DSC)

The degree of starch gelatinization (DG) in the baked samples was measured using a Mettler Toledo 821e DSC (Mettler-Toledo Inc., USA), according to Contardo et al. (2016) with some adjustments. The dough was first dehydrated at 45°C until it reached the same moisture content that biscuits attained after baking. Then, the ground sample (~15 mg, either raw dough or baked biscuit) was placed into a 160 µL aluminum pan, and distilled water was added to yield a water-to-sample ratio of 4:1. The pans were hermetically sealed and equilibrated at room temperature for 25 h prior to the analysis. A hermetically sealed pan containing distilled water (the same amount used for the sample preparation) was used as a reference. Finally, the samples were heated from 25°C to 90°C at 5°C/min, and the DG was derived in triplicate from the enthalpy values of raw ($\Delta H_{dough}, J/g$) and baked ($\Delta H_{biscuit}, J/g$) sample, according to **Eq. (3.2)**:

$$DG(\%) = \left(\frac{\Delta H_{dough} - \Delta H_{biscuit}}{\Delta H_{dough}}\right) \cdot 100 \tag{3.2}$$

### 3.2.6 Scanning electron microscopy (SEM)

Prior to the SEM analysis, the biscuits were defatted at room temperature following the procedure described by Pareyt et al. (2010a) with some modifications. The entire sample was then immersed in petroleum ether (50ml) for 30 min. The solvent was then replaced, and the defatting procedure was repeated seven times. Defatted biscuits were mounted in a SEM pin specimen holder with a carbon conductive adhesive tape and were coated with gold (~10 nm) using a 108Auto/SE Sputter Coater with MTM-20 high-resolution thickness controller (Ted Pella, INC., USA). Finally, the samples were examined using Quanta™ 250 FEG-SEM (Thermo Fisher Scientific, USA), at an accelerating potential of 20 kV, using magnifications ranging from 600× to 1300×.

### 3.2.7 X-ray micro-computed tomography (X-ray μCT)

***Image acquisition and reconstruction***

The microstructure of rotary-moulded biscuits was further characterized using a Skyscan 1272 X-ray micro-computed tomography system (version 1.1.7, Bruker Corp., Belgium), where the X-ray source operated at a voltage of 45 kV and a current of 222 μA. Images were acquired using an exposure time of 480ms, over an interval of 0-360° with a rotation step of 0.2°, and two frame averaging. Three samples were scanned for each condition.

Around 1270 projection images were obtained from the image acquisition, which were then processed using reconstruction software (NRecon v. 1.7.3, Bruker Corp., Belgium) to obtain 2D cross-sectional images (resolution 2016 × 1344 pixels, voxel size of 10.7μm × 10.7μm × 10.7μm). During the reconstruction phase, the following parameters were set to obtain a good quality of the reconstructed images: *thermal correction* (X/Y alignment with a reference scan), *misalignment compensation* (post-alignment), *smoothing* (1, using Gaussian Kernel = 2), *ring artifacts reduction* (= 5), and *beam-hardening correction* (= 45%).

***Image processing and analysis***

The reconstructed images were processed and analyzed using CTAn software (version 1.17, Bruker Corp., Belgium), which is made up of the following steps: (i) selection of a volume of interest (VOI) in order to reduce the time consumption during the 3D quantification; (ii) removal of residual noise by mainly using global threshold, despeckle, and bitwise operations; (iii) the definition of a region of interest (ROI) in order to establish the boundary limits of the object prior to segmentation; (iv) the segmentation of biscuit components (biscuit matrix and air pores) by applying bitwise operations between the ROI and the binary image; and finally, (v) three-dimensional quantification using the structure thickness methodology, which enables a 3D representation of the size distributions of biscuit components to be obtained. This method measures the local thickness of the 3D structure in two steps. First, a skeletonization step is applied to obtain the medial axes of the structure. Then, a sphere-fitting local thickness is used to fill the structure with the largest sphere at any point of the medial axis (Bruker, 2019).

### 3.2.8 Sensory analysis

The sensory attributes of biscuits were analyzed with 11 trained panelists using the monadic profiling technique. The intensity of the grittiness and the hardness was evaluated in two assessments, as recommended by Meyners et al. (2020) and Moser et al. (2018). An eleven-point hedonic scale was then used during the evaluation, from 0 to 10, where the intensity ranges were defined as follows: 0.1 to 2 (weak); 2.1 to 4 (slightly weak); 4.1 to 6 (moderate); 6.1 to 8 (slightly strong); and 8.1 to 10 (strong).

The sweetness of biscuits was measured using a discrete point time intensity test, with the purpose of identifying changes in sweetness intensity during the oral processing phase. The sweetness intensity scale was defined from 0 to 10 (0=none; 5=moderate; 10=strong). Eight training sessions were required with the panelists in order to define and standardize the test conditions, practice with the software interface, and to gain confidence with the entire protocol. This procedure followed all the recommended steps by Lawless & Heymann (2010) for the purpose of carrying out a time–intensity study. During the preliminary sessions, the chewing time required for half of a biscuit was defined, which was 20 s. The interval time between each measurement during mastication was also specified, which was every 5 s. Furthermore, a residual sweetness intensity was included after 10 s of the sample being swallowed. During the test, each panelist inserted the sample into their mouth and the sweetness intensity at 5, 10, 15 and 20 s of chewing, and after 10 s of being swallowed, was recorded using the FIZZ software (version 5.2, Biosystemes, France). After consuming each sample, panelists were instructed to rinse their mouths by drinking water and eating some plain crackers, as suggested by Kohyama et al. (2016) and Monteleone et al. (2014), and to rest for 15 min before taking the following sample test. A total of six sessions were required to conduct the whole experiment.

### 3.2.9 Statistical analysis

The experimental data were analyzed with R (R Foundation for Statistical Computing, United States), version 3.6.1. With respect to the statistical analysis as to whether there was any difference between each of the treatment means, one-way Welch's ANOVA was applied when $n \geq 30$, when analyzing biscuit dimensions. This test is used when homoscedasticity cannot be assumed, but normally distributed data is required. The assumption of normality is not contradicted, as the Central Limit Theorem approximation is sufficient if the sample size is greater than 30 (Navidi, 2011). The pairwise post-hoc analysis was performed using the Games-Howell test at 95% confidence, which does not assume homoscedasticity and equal sample sizes (Games et al., 1979). However, when $n < 30$, the bootstrap method introduced by Efron (1992) was used as it does not make any assumption on the underlying population distribution (*i.e.* a non-parametric approach) (Rizzo, 2007). In particular, the analysis performed for this study did not assume that the data came from a normal distribution and instead fully accepted that the population distribution is unknown. The bootstrap method uses sampling with replacement for the existing data to recover the unknown population distribution of the parameters of interest (in this case *mean*) and to estimate confidence intervals. To this effect, one thousand replicates were created, and 95% confidence intervals were obtained to test differences between the means of the different formulations.

## 3.3 Results and Discussion

### 3.3.1 Distribution of sucrose particle size

Characterization of the particle size distribution of granulated or powdered sucrose is a first critical step towards understanding the role of sugar crystal size during the processing of rotary-moulded biscuits. Both granulated and powdered sucrose presented unimodal distributions. However, 90% of particles ($D_{90}$, based on a volume distribution) had a diameter of or below 978 µm in granulated sucrose, whereas this value dropped to 98 µm in powdered sucrose. In addition to this, the surface-area-to-volume ratio (1/mm) was calculated according to **Eq. (3.1)** to have an approximation to the specific surface area of sucrose particles. The surface-area-to-volume ratio was 6.7 (1/mm) in granulated sucrose and 28.1 (1/mm) in powdered sucrose. These results suggest that powdered sucrose should dissolve much faster in water, as its surface-area-to-volume ratio is approximately four-times higher than that of granulated sucrose.

### 3.3.2 Vertical expansion of rotary-moulded dough during baking

**Figure 3.1** shows the vertical expansion of biscuit doughs produced with either granulated or powdered sucrose using different sucrose:flour ratios (d.b.), as a function of the baking time (s). Overall, it is possible to observe that the doughs expanded until they reached a maximum height, and then most of them underwent a vertical collapse as baking progressed. An exception was observed at the highest sucrose:flour ratio (**Figure 3.1.A**). The biscuit dough produced with powdered sucrose at the maximum concentration level never collapsed. Moreover, it achieved an expanded inner structure (10.3 ± 0.7 mm) until the end of baking, whereas the sample with granulated sugar obtained a maximum height of 9.4 ± 0.9 mm, and then the dough collapsed. These values were higher than those obtained at lower sucrose:flour ratios when using either granulated or powdered sucrose, which were 8.1 ± 0.3 or 8.0 ± 0.9 mm, 7.1 ± 0.4 or 6.7 ± 0.7 mm, and 5.2 ± 0.4 or 5.6 ± 0.6 mm at ratios of 0.5, 0.3 or 0.1, respectively (**Figures 3.1.B, 3.1.C, and 3.1.D**). Furthermore, doughs with the highest sucrose concentration expanded for a longer time (240 s) during baking compared to those with lower sucrose:flour ratios, whose maximum expansion was achieved at 120, 100 and 60 s for ratios of 0.5, 0.3 and 0.1, respectively. These results show that the reduction of sucrose content limits the expansion of rotary-moulded biscuits, speeding-up the expansion peak and reducing the maximum height.

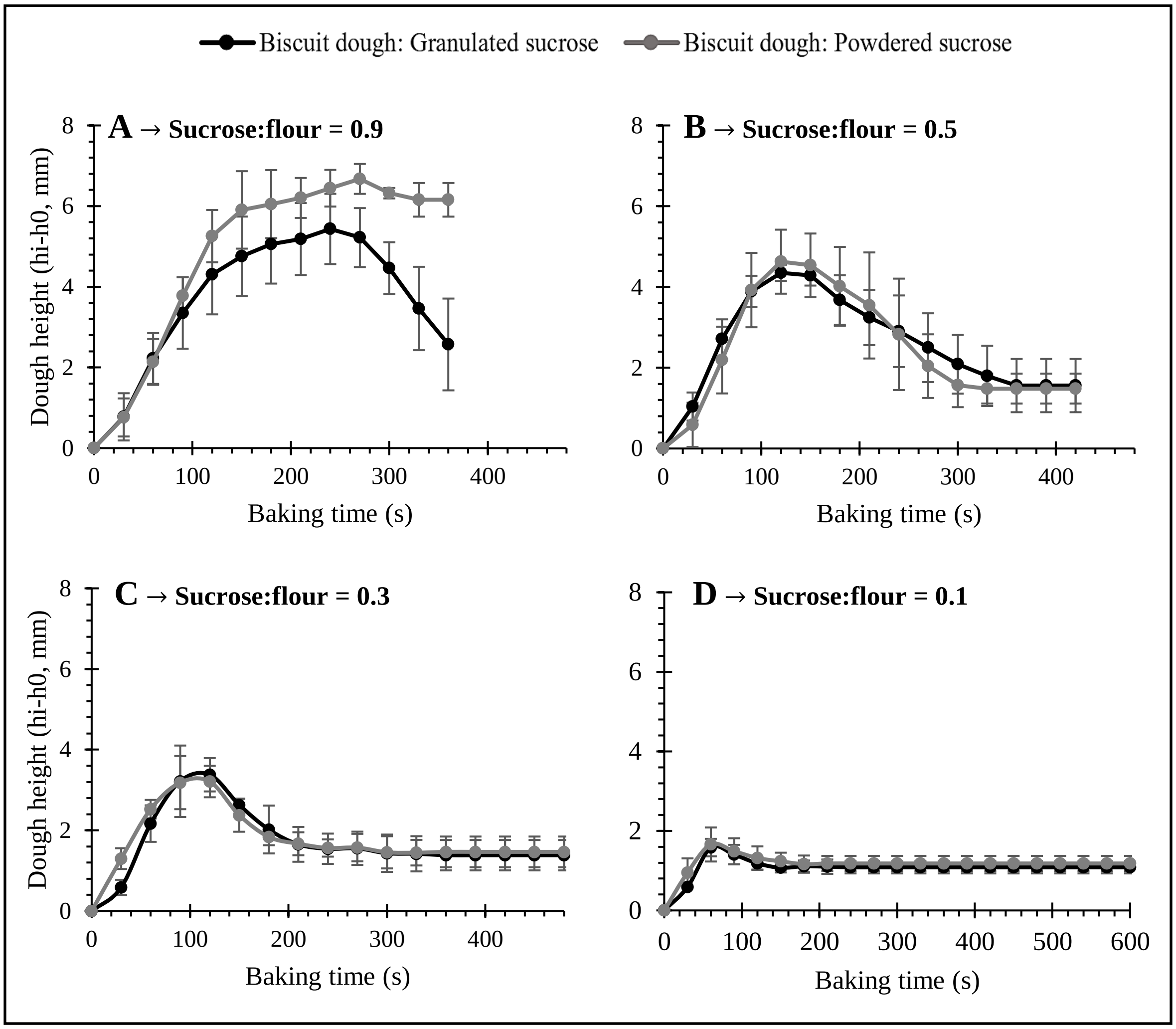


**Figure 3.1** Vertical expansion of biscuit dough prepared with either granulated (black curve) or powdered sucrose (grey curve) using sucrose:flour (g/g d.b.) ratios of 0.9 (A), 0.5 (B), 0.3 (C) or 0.1 (D), measured by time-lapse photography. It is expressed as $(\boldsymbol{h_o} - \boldsymbol{h_i})$, where $\boldsymbol{h_o}$ and $\boldsymbol{h_i}$ is the height of the dough at baking time $\boldsymbol{t = 0}$ (3.5 mm) or $\boldsymbol{t = i}$. Data are means ± standard deviation (n = 3).

### 3.3.3 Dimensions and aeration after baking of rotary-moulded biscuits

The biscuit dimensions were measured after baking, and the results are presented in **Table 3.1**. A significant higher value (p-value < 0.05) was obtained in the thickness of biscuits at the highest sucrose:flour ratio, either using powdered sucrose (10.98 mm) or granulated sugar (5.76 mm). However, this dimension practically did not change when decreasing the sucrose:flour ratio from 0.5 to 0.1. A significant reduction of only ~3% was observed in biscuits prepared with powdered sucrose with a 0.1 ratio, compared to those prepared with a 0.5 ratio. The horizontal dimensions are linked to the lateral spread of the dough from its original size ($40 \times 40$ $mm^2$). In biscuits with granulated sucrose, the reduction of the sucrose:flour ratio significantly decreased (p-value < 0.05) the length and width. In samples formulated with powdered sucrose, the length and width were similar at ratios of 0.9 and 0.5, however they decreased as the sucrose:flour ratio was further reduced to 0.3 and 0.1. In addition, at ratios of 0.9 or 0.5, biscuits laterally spread between ~1 to ~3 mm relative to their original size. No changes were observed in biscuits with a sucrose:flour ratio of 0.3 and, conversely, a noticeable reduction in length was observed in biscuits prepared with a ratio of 0.1.

Regarding the effect of sucrose particle size on dimensions, significant differences (p-value < 0.05) were observed at sucrose:flour ratios of 0.9 and 0.5. Some significant (p-value < 0.05) but minor changes in thickness and length were observed in biscuits with a ratio of 0.3, whereas no significant differences were observed in samples prepared with a sucrose:flour ratio of 0.1. Pareyt & Delcour (2008a) and Kweon et al. (2009) mentioned that in sugar-snap or wire-cut biscuits, coarser sugar crystals produce a smaller biscuit diameter compared to a biscuit with finer crystals. On the basis of our results, a similar behavior was observed in biscuits with sucrose:flour ratios of 0.9 or 0.5, and to some extent in biscuits with a ratio of 0.3, which disappears at the lowest concentration level.

The vertical expansion of biscuit dough during baking is needed to develop the desired aerated structure upon baking. To better understand this phenomenon, the porous microstructure of rotary-moulded biscuits was characterized by means of X-ray micro-CT. **Table 3.1** shows the air porosity (%) of each sample that was measured out of 3D micro-CT images through the quantification of the volume of voxels corresponding to air pores over the total volume of voxels. The highest porosity was observed in samples with the greatest sucrose:flour ratio, where the air porosity of the non-collapsed biscuit (~80%), which was formulated with powdered sucrose, was significantly higher (p-value < 0.05) than the one prepared with granulated sucrose (~61%). A further reduction of the sucrose:flour ratio from 0.5 to 0.1 slightly affected the air porosity, and a maximum variation of 3% was observed among these samples.

**Table 3.1** Dimensions, air porosity, and sensory attributes (grittiness and hardness at first bite) of rotary-moulded biscuits prepared with either granulated (GS) or powdered sucrose (PS) using sucrose:flour (g/g d.b.) ratios of 0.9, 0.5, 0.3 or 0.1. Data are means ± confidence interval at 95%. Different scripts per parameter denote significant differences ($p < 0.05$).

| Sucrose:flour ratios (g/g) | Biscuit Dimensions (mm) | | | Air porosity (%) (Micro-CT) | Grittiness (Sensory) | Hardness First Bite (Sensory) |
|---|---|---|---|---|---|---|
| | Thickness | Length | Width | | | |
| 0.9 GS | $5.76 \pm 0.08^{d}$ | $43.40 \pm 0.27^{f}$ | $43.33 \pm 0.23^{e}$ | $61.59 \pm 1.97^{c}$ | $6.5 \pm 0.3^{d}$ | $5.5 \pm 0.2^{d}$ |
| 0.9 PS | $10.98 \pm 0.20^{e}$ | $42.86 \pm 0.20^{e}$ | $42.43 \pm 0.22^{d}$ | $80.18 \pm 0.61^{d}$ | $6.6 \pm 0.3^{d}$ | $5.4 \pm 0.2^{d}$ |
| 0.5 GS | $4.92 \pm 0.06^{a}$ | $41.09 \pm 0.13^{d}$ | $41.00 \pm 0.09^{c}$ | $56.44 \pm 2.22^{ab}$ | $5.9 \pm 0.5^{c}$ | $5.6 \pm 0.3^{d}$ |
| 0.5 PS | $5.21 \pm 0.05^{c}$ | $43.32 \pm 0.22^{ef}$ | $42.70 \pm 0.15^{d}$ | $57.26 \pm 0.79^{b}$ | $4.5 \pm 0.7^{b}$ | $5.5 \pm 0.3^{d}$ |
| 0.3 GS | $4.90 \pm 0.05^{a}$ | $40.56 \pm 0.13^{b}$ | $39.98 \pm 0.96^{abc}$ | $59.71 \pm 0.24^{c}$ | $0.1 \pm 0.1^{a}$ | $4.9 \pm 0.3^{c}$ |
| 0.3 PS | $5.11 \pm 0.04^{bc}$ | $40.15 \pm 0.17^{c}$ | $40.40 \pm 0.09^{b}$ | $57.19 \pm 0.46^{b}$ | $0.1 \pm 0.1^{a}$ | $4.8 \pm 0.2^{bc}$ |
| 0.1 GS | $4.96 \pm 0.09^{ab}$ | $37.19 \pm 0.19^{a}$ | $39.82 \pm 0.19^{a}$ | $56.01 \pm 1.52^{b}$ | $0.0 \pm 0.0^{a}$ | $4.5 \pm 0.2^{ab}$ |
| 0.1 PS | $5.05 \pm 0.08^{ab}$ | $36.81 \pm 0.22^{a}$ | $39.73 \pm 0.14^{a}$ | $54.09 \pm 1.21^{a}$ | $0.0 \pm 0.0^{a}$ | $4.4 \pm 0.1^{a}$ |

To further understand the effect of sucrose reduction in biscuit porosity, the size distribution of air pores was analyzed. A 3D representation of the air pores is shown in **Figure 3.2**, where each color represents a specific size-range of air pores, according to the color-bar legend. The smallest air pores (<500 µm) are represented by colors ranging from dark red to yellow, whereas the biggest ones (>1500 µm) are represented in green and white. In addition to this information, the structure thickness distribution of air pores was also obtained from micro-CT images analysis. This is a quantitative approach that represents the air pores size distribution, and the results are presented in **Figure 3.3**. It can be seen that the biggest pores only appeared in the structure of biscuits with the highest sucrose:flour ratio (**Figure 3.2.A**). In these biscuits, those prepared with granulated sucrose had ~89% of their air pores smaller than 867 µm. In turn, biscuits prepared with powdered sucrose only had 34% below this size and contained the largest air pore (>2033 µm), which represented ~62% of the total air (**Figure 3.3**). The non-collapsed structure that was observed in biscuits prepared with powdered sucrose at its maximum concentration level (**Figure 3.1.A**) can be explained by the existence of a single major central void that was preserved after baking (**Figure 3.2.A, right side**). Although similar values of total air porosity were obtained in biscuits with sucrose:flour ratios of 0.5, 0.3 and 0.1, important differences were observed with respect to their air-pores size distribution. A reduction of the sucrose:flour ratio increased the percentage of small air pores (11 to 267 µm range, in **Figure 3.3**), which corresponds to an increase in the dark red to dark orange scale in **Figures 3.2.B, 3.2.C and 3.2.D**. Biscuits with a sucrose:flour ratio of 0.5 and 0.3 had 70-75% of their air pores in the smallest size range, which is significantly lower ($p$-value < 0.05) than the 81-83% obtained in biscuits with a ratio of 0.1. The microstructure of biscuits with the lowest sugar concentration had the higher percentage of small pores and experienced the lowest vertical expansion during baking (**Figures 3.1.D**). Regarding sugar crystals, the sucrose particle size produced a marginal difference in air porosity in biscuits with a sucrose:flour ratio of 0.3 and 0.1 (**Table 3.1**), where the value of samples prepared with granulated sucrose was ~2% higher ($p$-value < 0.05) than that of those prepared with powdered sucrose. The results obtained by micro-CT are consistent with those reported by Pareyt et al. (2009a), who showed a decrease in the size of air pores when the sugar level of sugar-snap biscuits was reduced from 31.2 to 17.6% (w.b.), also finding that lower sugar levels decreased the rise of dough during baking, resulting in smaller mean air pore sizes.

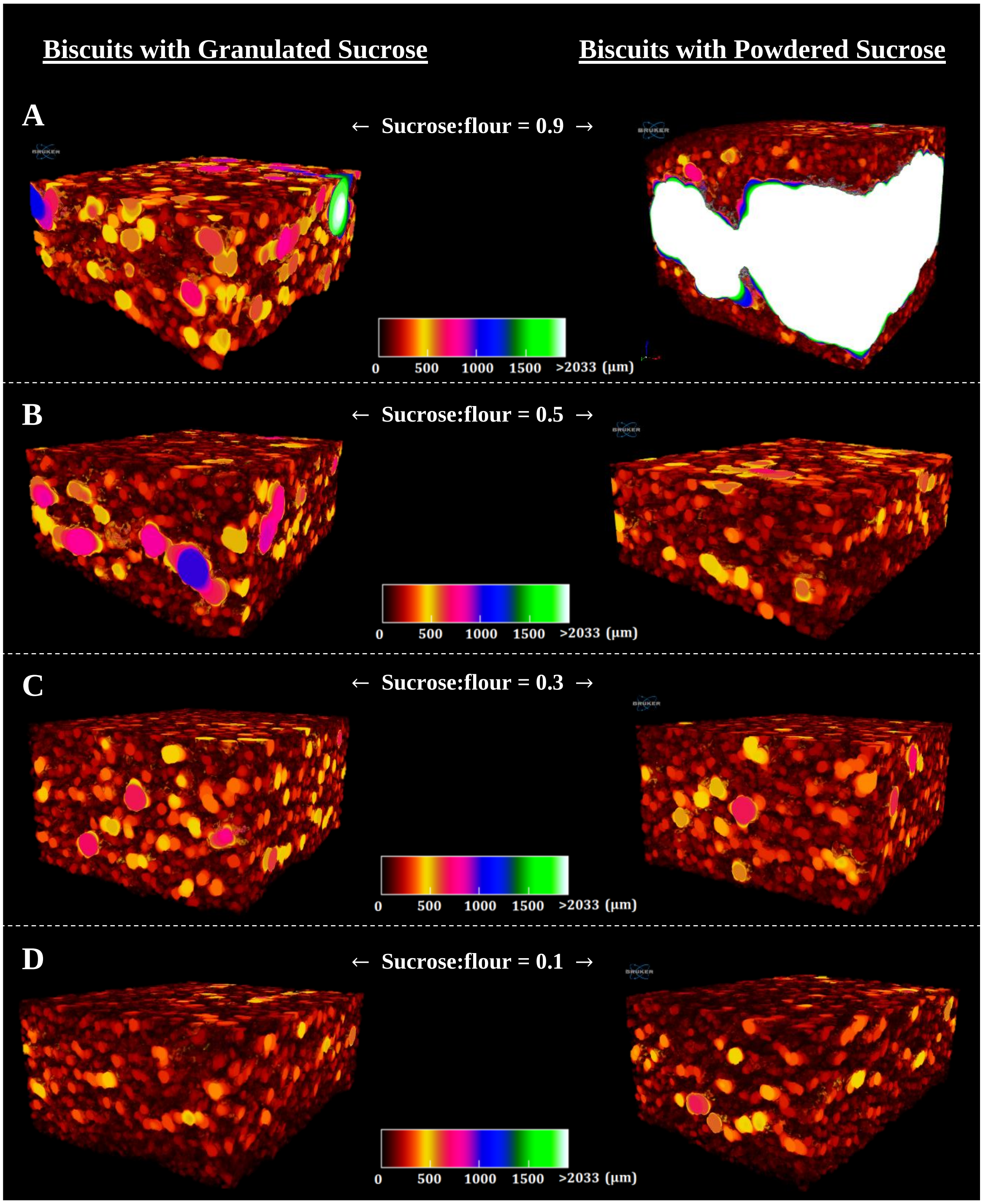


**Figure 3.2** 3D microtomography images of the air pores distributed within biscuits prepared with either granulated (left) or powdered sucrose (right) using sucrose:flour (g/g d.b.) ratios of 0.9 (A), 0.5 (B), 0.3 (C) or 0.1 (D). The air pore sizes are represented according to the color-bar legend, where dark red to orange colors represent the smallest air pores (<500µm), whereas white color represents the biggest air pores (>1905µm).

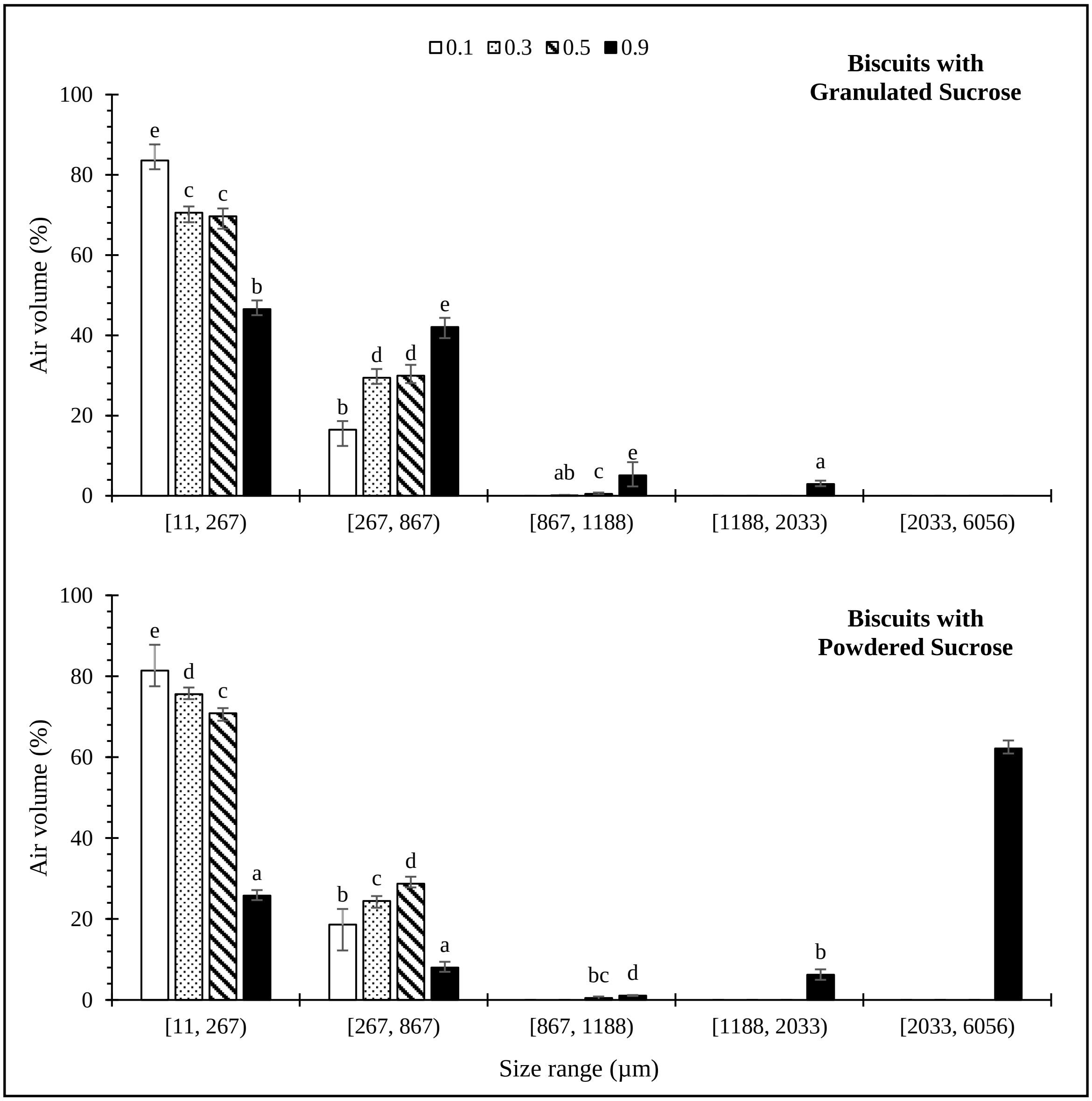


**Figure 3.3** Structure-thickness distribution of air pores inside the biscuit structure, which were prepared with either granulated (top figure) or powdered sucrose (bottom figure) using sucrose:flour (g/g d.b.) ratios of 0.9 (black bar), 0.5 (dashed bar), 0.3 (dotted bar) or 0.1 (white bar). Data are means ± confidence intervals at 95%. Different scripts per range interval denote significant differences ($p < 0.05$).

### 3.3.4 Starch gelatinization during baking

In order to understand the role of starch on the vertical expansion of rotary-moulded dough during baking, the degree of starch gelatinization (DG) was quantified. Results were determined according to **Eq. (3.2)** and are presented in **Figure 3.4**. Overall, partial starch gelatinization (mean values below 41%) was observed in all biscuits. These results are consistent with those reported by Canalis et al. (2018) and Laguna et al. (2013a), who obtained partially gelatinized starch in sugar-snap biscuits when the sucrose content was reduced from 27% to 15% (w.b.), or from 17% to 0% (w.b.). It can also be observed that even though DG mean values were slightly lower in biscuits elaborated with powdered sucrose, no significant differences (p-value > 0.05) were observed in biscuits with different sucrose particle sizes, except for biscuits prepared with a sucrose:flour ratio of 0.3. With respect to the effect of sugar concentration, a significantly higher DG (p-value < 0.05) was observed as the sucrose:flour ratio was reduced. The lowest DG was obtained in biscuits with a sucrose:flour ratio of 0.9, where only 7.96% and 5.93% gelatinization occurred in biscuits prepared with either granulated or powdered sucrose, respectively. These values increased up to 41.10 and 40.14% in biscuits prepared with a sucrose:flour ratio of 0.1.

These results agree with observations made using SEM, which showed a vast amount of ungelatinized starch granules in biscuit cross sections, as shown in **Figure 3.5**. In biscuits with the highest sucrose:flour ratio (**Figure 3.5.A-B**), a smooth film surrounding the biscuit structure can be observed, which may correspond to amorphous sucrose obtained after water evaporation from a highly concentrated sucrose solution during baking. The film appears to be more continuous in biscuits formulated with powdered sucrose (**Figure 3.5.B**), compared to those prepared with granulated sucrose (**Figure 3.5.A**), which may be due to the greater dissolution rate of smaller sucrose crystals compared to coarser ones. The smooth surface was also found in some areas of biscuits prepared with a ratio of 0.5 (Figure **3.5.C-D**), which was not observed at lower sucrose:flour ratios (**Figure 3.5.E to 3.5.H**). At ratios of 0.3 or 0.1, some starch granules had a more irregular shape, with a depressed center and lower sphericity. This may be associated with the morphological changes that occur during gelatinization (Molina et al., 2016).

Reduced starch gelatinization in dough with a high sucrose content may be due to the antiplasticizing effect of the sucrose-water cosolvent, which retards the sequential thermal events of gelatinization (Slade & Levine, 1994). When analyzing the sucrose-to-added-water ratio in the different doughs, this value was found to be 6.2, 4.1, 1.8 and 0.7 $\frac{g\ sucrose}{g\ water}$ in samples with sucrose:flour ratios of 0.9, 0.5, 0.3 and 0.1, respectively. During mixing, part of the sugar crystals will dissolve, whereas a fraction will remain as crystals. As sucrose solubility increases with temperature, from 2.07 $\frac{g\ sucrose}{g\ water}$ (~67% w/w) at 25°C to 4.76 $\frac{g\ sucrose}{g\ water}$ (~82% w/w) at 100°C (Bubník & Kadlec, 1995), sucrose crystals will continue to dissolve during baking, and it is likely that finer crystals will dissolve faster than coarser ones due to their higher surface-area-to-volume ratio, as discussed in section 3.3.1. With respect to the volume of the resultant sucrose solution, Ghiasi & RC (1983) found that when one gram of sucrose is dissolved in one gram of water, the volume of the solution increases by 0.6 ml. Accordingly, sucrose dissolution may impart mobility to the dough, particularly during the early stages of baking, allowing the dough to better spread and expand, because of the greater solution volume obtained due to sucrose dissolution (van der Sman et al., 2018; Davis, 1995; Curley & Hoseney, 1984). However, it has also been shown that, depending on the concentration of the sugar solution, this solution may affect the plasticization of starch granules compared to water alone, increasing the gelatinization temperature (Kweon et al., 2014; Perry & Donald, 2002; Slade & Levine, 1994; Slade & Levine, 1987). Consequently, the low degree of gelatinized starch obtained in doughs with high sugar concentrations may be due to the anti-plasticizing effect of the sucrose-water solution, which would retard the sequential thermal events of gelatinization (Slade & Levine, 1994). This aspect may also affect the thermal transitions of gluten proteins, as pointed out by Pareyt et al. (2009b), and consequently hence the mobility of dough during baking. The expansion of dough is described by being lateral and vertical, where vertical expansion is more pronounced than horizontal spread mainly due to the effect of the leavening agents. A semi-rigid structure is needed to control and withstand the lateral spread and the vertical expansion produced by the steam pressure and the carbon dioxide produced by chemical leaveners. Controlling the dough expansion has been mainly attributed to starch gelatinization and the denaturation of gluten proteins, which become insoluble as aggregation/cross-linking occurs above 85°C (Pareyt et al., 2008b;

Chevallier et al., 2002; Slade & Levine, 1994). Pareyt et al. (2009b) concluded that high levels of sucrose in sugar-snap dough (26.9% to 39.5%, w.b.) delayed the entanglement and/or cross-linking of gluten proteins, thus postponing the dough setting and allowing it to expand for a longer time period during baking. From our results **(Figure 3.1**), it can be observed that the dough sets (reaches the maximum level of expansion) later as the sucrose:flour ratio increases. Additional studies are certainly needed to further understand the influence of the entanglement of gluten proteins over starch gelatinization on structure formation and the expansion of rotary-moulded dough during the baking process.

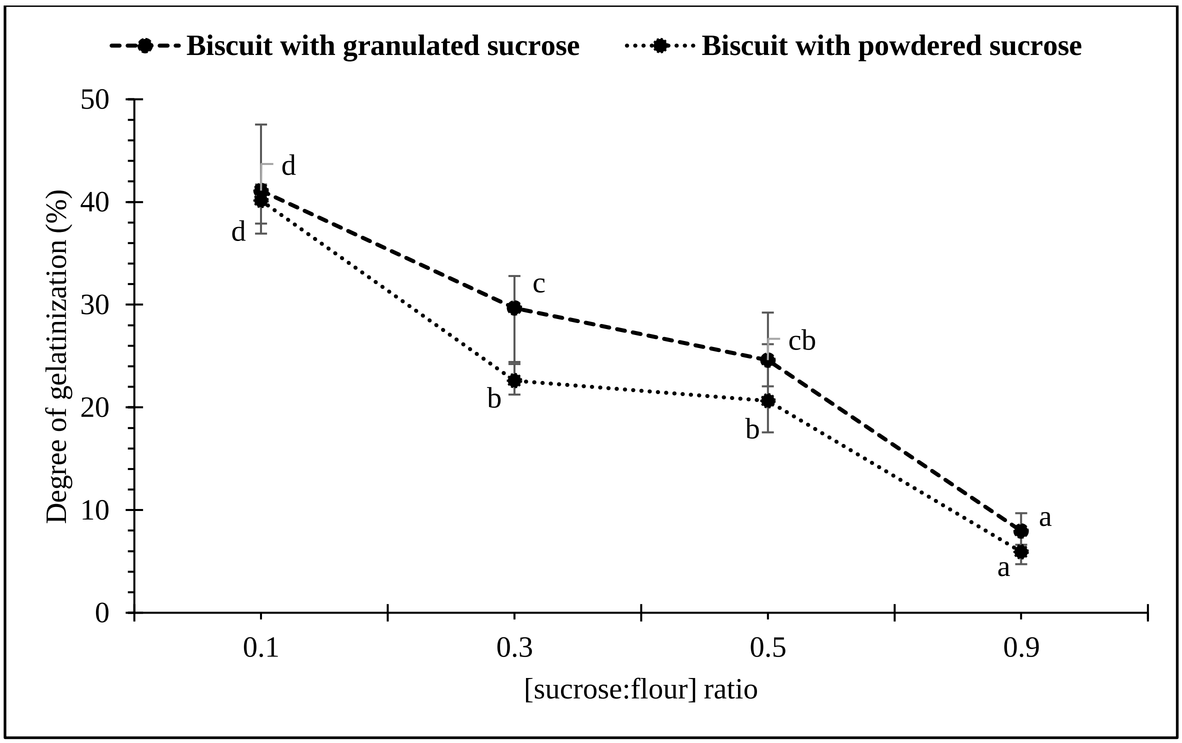


**Figure 3.4** Degree of starch gelatinization (%), obtained by DSC, of biscuits prepared with either granulated (dashed line) or powdered sucrose (dotted line) using sucrose:flour (g/g d.b.) ratios of 0.9, 0.5, 0.3 or 0.1. Data are means ± confidence intervals at 95%. Different scripts denote significant differences ($p < 0.05$).

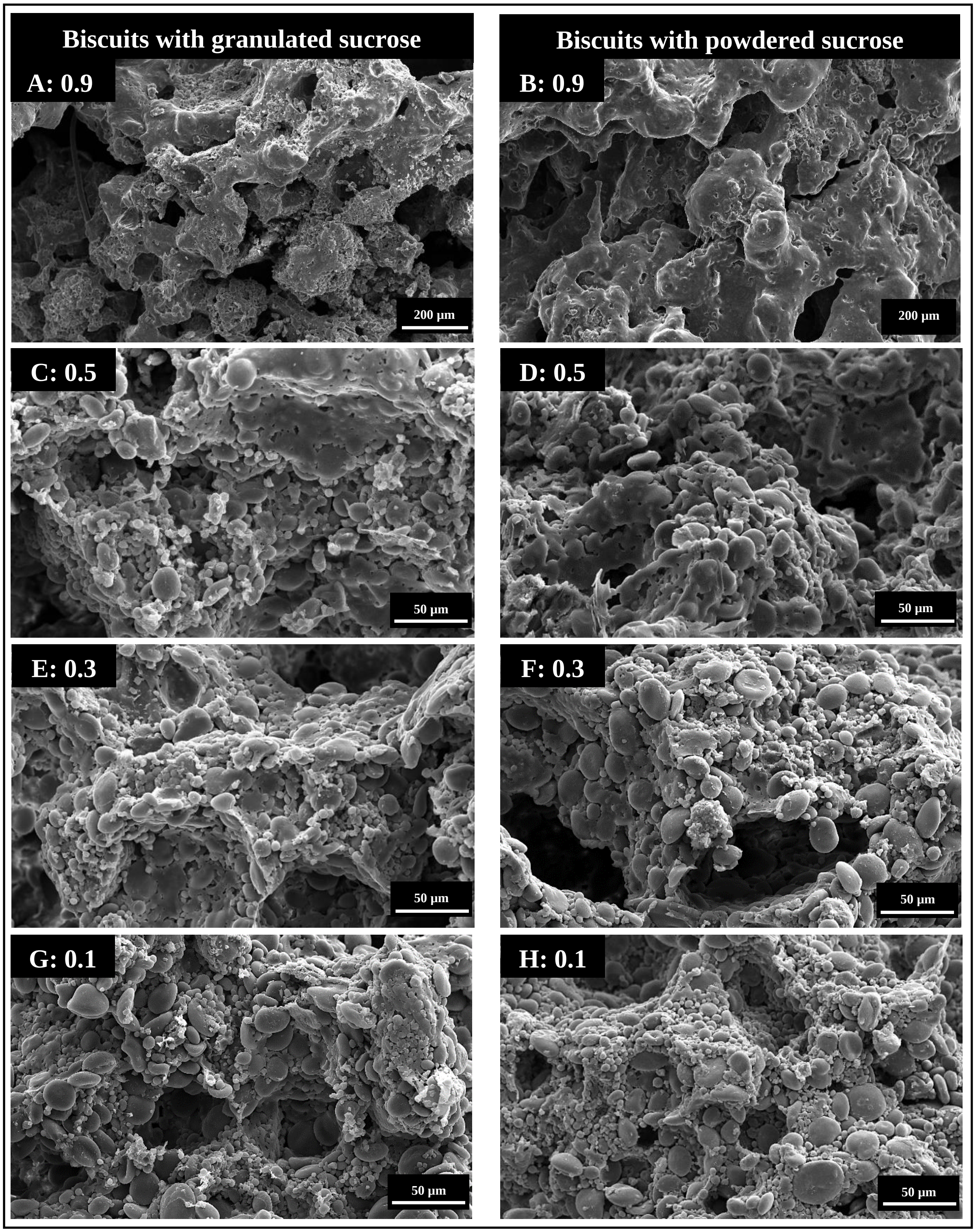


**Figure 3.5** Cross-sectional scanning electron micrographs (magnification of 1300× or 300×) of biscuits prepared with either granulated or powdered sucrose using sucrose:flour (g/g d.b.) ratios of 0.9 (A, B), 0.5 (C, D), 0.3 (E, F) or 0.1 (G, H).

### 3.3.5 Sensory attributes and their relationship to microstructural parameters

The relationship between sensory attributes and biscuit formulation is certainly an important aspect to consider. In this study, the hardness and the grittiness of rotary-moulded biscuits were evaluated with a trained sensory panel using an 11-point hedonic scale. The hardness was defined by panelists as the force required to break the biscuit after the first bite with incisors, and it was examined because previous studies have shown that biscuit hardness (measured using a texture analyzer) is affected by sugar reduction (Canalis et al., 2018; Laguna et al., 2013a; Maache-Rezzoug et al., 1998). In addition, grittiness was defined as the perception of particles between the tongue and palate or the tongue and teeth, and panelists have associated this attribute to the presence of sugar crystals during biscuit chewing. As shown in **Table 3.1**, samples prepared with a sucrose:flour ratio of 0.9 or 0.5 were much grittier than those prepared with 0.3 or 0.1, which were actually not perceived as gritty, thus reflecting a relevant sucrose concentration threshold for grittiness perception. No significant differences in grittiness between biscuits prepared with granulated sucrose or powdered sucrose were perceived, except from those prepared with a sucrose:flour ratio of 0.5, where those prepared with granulated sucrose were perceived as grittier ($p$-value $< 0.05$).

The reduction of sucrose:flour ratio from 0.9 to 0.5 significantly decreased ($p$-value $< 0.05$) the grittiness. This perception may be related to the presence of large undissolved sugar crystals, as observed in samples prepared with granulated sucrose, as shown in **Figures 3.6.A and 3.6.B**, which presents X-Y cross-sectional micro-CT images of biscuits (where the biscuit matrix is represented in orange-to-yellow colors and the air pores are shown in black). Coarse sucrose crystals could be recognized in micro-CT images due to their morphology. They may have remained as such, due to insufficient hydration and dissolution during baking, as previously observed by Chevallier et al. (2002), who found crystalline sucrose on the surface of biscuits with 27% (d.b.) sucrose, a similar concentration to biscuits prepared with a sucrose:flour ratio of 0.5. Smaller crystals could not be identified, as they overlapped with the matrix in the gray scale histogram (Contardo et al., 2018). However, rather thicker matrix regions were observed in biscuits prepared with granulated or powdered sucrose at ratios of 0.9 or 0.5, compared to those prepared with sucrose:flour ratios of 0.3 or 0.1, which may be related to the higher grittiness perception.

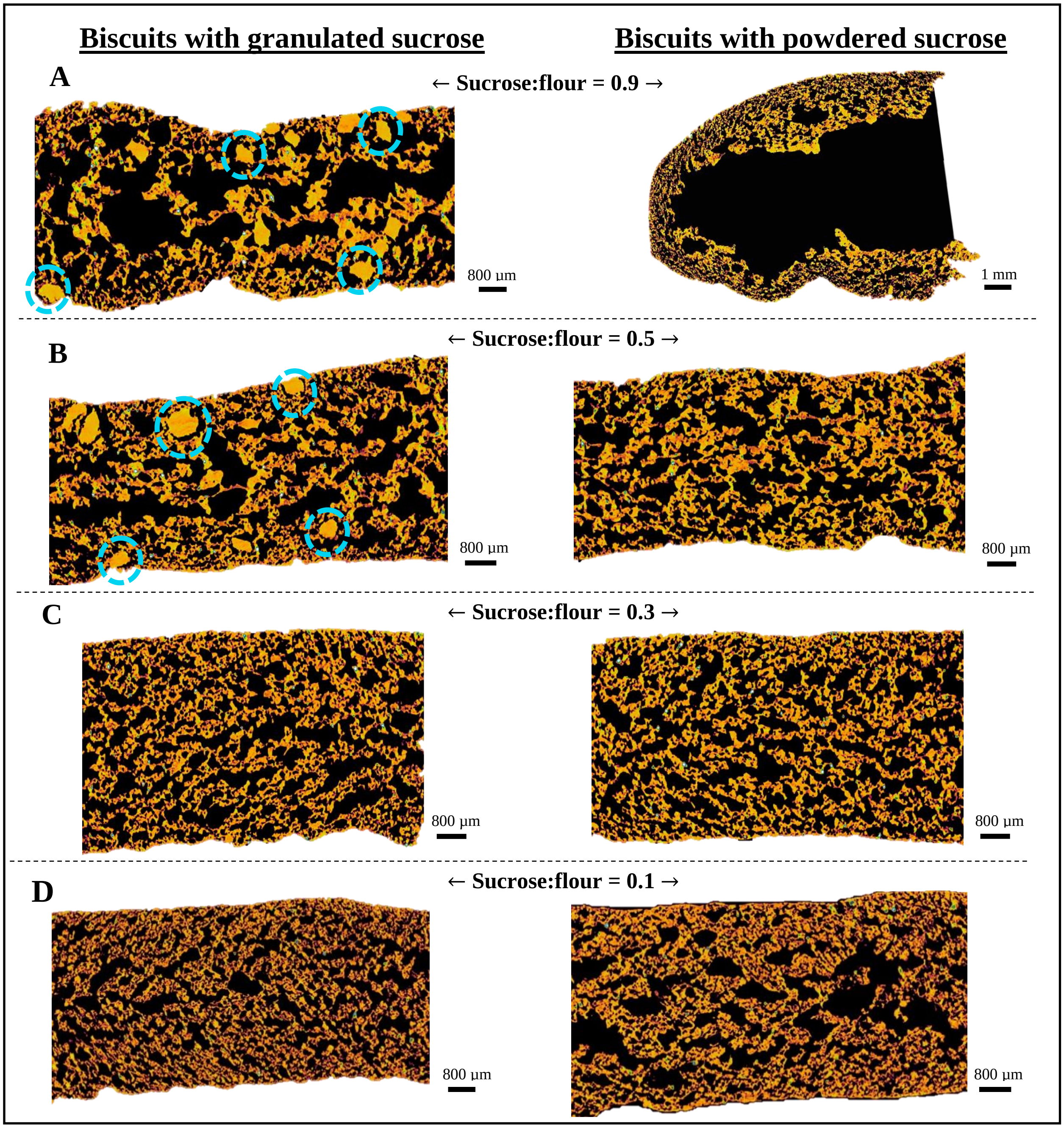


**Figure 3.6** 2D X-Y cross-sectional images (obtained by X-ray µCT) of biscuits prepared with either granulated (left) or powdered sucrose (right) using sucrose:flour (g/g d.b.) ratios of 0.9 (A), 0.5 (B), 0.3 (C) or 0.1 (D). Sucrose crystals are identified in biscuits prepared with granulated sucrose using sucrose:flour ratios of 0.9 or 0.5.

To further understand this effect, the wall thickness distribution of the biscuit matrix was quantified (3D quantification), as shown in **Figure 3.7**. It can be observed that the wall's cumulative distribution decreased as sucrose content increased, showing that higher sucrose content biscuits had a higher structure of thickness. This may be related to a perception of increased grittiness, but also to the greater hardness at the first bite, as seen in **Table 3.1**. Results showed that samples with sucrose:flour ratios of 0.9 and 0.5 were significantly harder than those prepared with 0.3 or 0.1 ratios, which exhibited much higher cumulative distribution percentages at lower size ranges. These results concur with those reported by Canalis et al. (2018) and Laguna et al. (2013a), who observed a decrease in hardness in sugar-snap biscuits when the sucrose content fell from 27% to 15% (w.b.) or from 17% to 0% (w.b.). The microstructural assessment related to the wall thickness distribution is consistent with results reported by Pareyt et al. (2009a), who showed a reduction of the mean wall thickness, and a strong association with the lower break strength (corrected for density) of sugar-snap biscuits, when the sugar level was decreased from 25.7% to 17.6% (w.b.).

In order to understand the effect of sucrose particle size during chewing, a methodology was implemented for sweetness perception, which was based on the criteria of a discrete point time intensity test. Results are presented in **Figure 3.8**, where sweetness intensity (from 0 to 10) of biscuits was measured during 20 s of chewing and then after 10 s of swallowing the sample (residual taste). Overall, the sweetness perception followed a quadratic behavior during chewing, where the maximum sweetness intensity was perceived at 19 or 20 s, that is, just before swallowing. The reduction of the sucrose:flour ratio significantly decreased ($p$-value $< 0.05$) the sweetness intensity during oral processing. The maximum value achieved on each curve was as follows: 8.0 (strong intensity) for biscuits with a sucrose:flour ratio of 0.9 (**Figure 3.8.A**); 6.5 (slightly strong intensity) for those with a ratio of 0.5 (**Figure 3.8.B**); 4.5 (moderate intensity) for samples with a ratio of 0.3 (**Figure 3.8.C**); and 2.2 (slightly weak intensity) for biscuits with the lowest sucrose:flour ratio (**Figure 3.8.D**). However, the sucrose particle size did not modify ($p$-value $> 0.05$) the intensity of sweetness perception during the mastication of rotary-moulded biscuits.

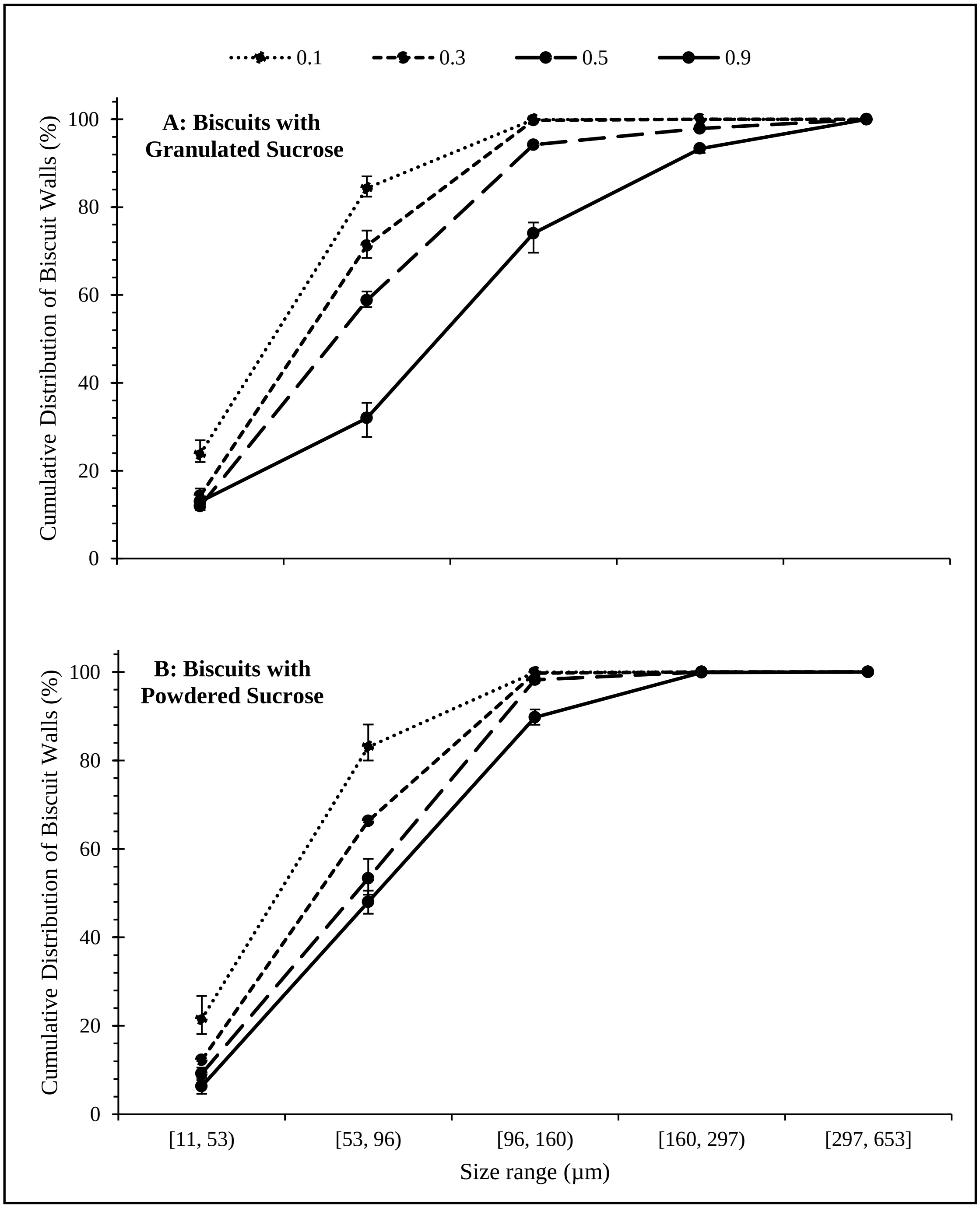


**Figure 3.7** Structure thickness distribution of the biscuits walls, which were prepared with either granulated (A) or powdered sucrose (B) using sucrose:flour (g/g d.b.) ratios of 0.9 (continuous line), 0.5 (long dashed line), 0.3 (short dashed line) or 0.1 (dotted line). Data are means ± confidence intervals at 95%.

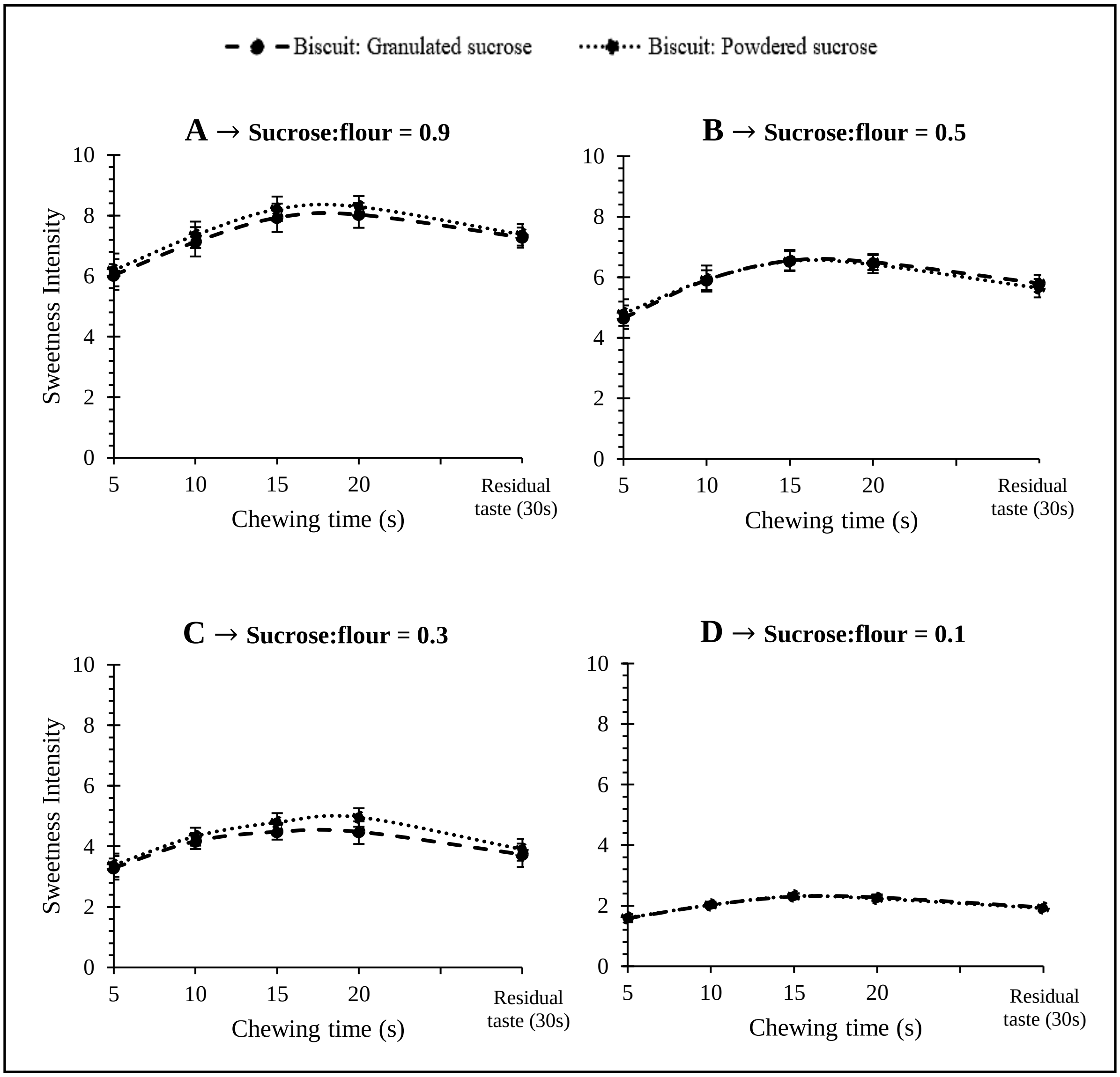


**Figure 3.8** Sweetness perception during mastication of biscuits prepared with either granulated (dashed line) or powdered sucrose (dotted line) using sucrose:flour (g/g d.b.) ratios of 0.9 (A), 0.5 (B), 0.3 (C) or 0.1 (D). Data are means ± confidence intervals at 95%.

## 3.4 Conclusion

Rotary-moulded biscuits have not been previously well characterized, as most of the studies have been focused on wire-cut or sugar-snap biscuits. This study revealed that dough expansion, starch gelatinization, microstructure and the sensory attributes of rotary-moulded biscuits were significantly affected by reducing the sucrose:flour ratio. The micro-CT analysis revealed that the size of air pores and the thickness of biscuit walls were reduced as the sucrose:flour ratio decreased. Additionally, sensory attributes (*i.e.* grittiness, hardness and sweetness perception) were detrimentally affected by decreasing the sucrose:flour ratio. However, the sucrose crystal size did not alter the intensity of sweetness perception during oral processing.

Overall, it was possible to confirm the occurrence of partial starch gelatinization in all the formulations that were studied, and it was found that the degree of gelatinization showed an inverse relationship with sugar concentration. It was also interesting to note that at the highest sucrose:flour ratio, an expanded and non-collapsing structure was obtained when the biscuit was formulated with powdered sucrose. This behavior could be linked to the low degree of starch gelatinization, but additional studies are certainly needed to understand this effect.

## 3.5 Acknowledgements

The authors are thankful to Katherine Allel and Michela Lauriola for their technical assistance in the sensory analysis, and to Conor McKee for performing the light scattering measurements. Furthermore, the authors would like to acknowledge the financial support of CONICYT through the Advanced National Human Capital Formation Program - Doctoral Fellowship 2016 (Folio n° 21161243), FONDEQUIP project EQM 130028, FONDEQUIP project EQM150101, and the NESTLÉ-UC Research Agreement.

### 3.6 Patent submission to the European Patent Office

The expanded, non-collapsed and aerated structure of rotary-moulded biscuit has not been seen before in this biscuit category, so that a patent was filed from this knowledge due to the feasible opportunity to produce a differentiated biscuit texture.

## Designation of inventor

User reference: 17366-EP-EPA
Application No:

Public

| | | | |
|---|---|---|---|
| | Inventor | | |
| | | Name: | MOLINA MAYDL María Teresa |
| | | Address: | San Josemaría Escriva de Balaguer 9423, apt. 505<br>Santiago<br>Chile |
| | | The applicant has acquired the right to the European patent: | As employer |

**Signature(s)**

Place:

Date: 09 April 2020

Signed by: /Stuart Lumsden/

Representative name: Stuart, Edward, Henry LUMSDEN

Capacity: (Representative 1)

Europäisches Patentamt
European Patent Office
Office européen des brevets

## Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

| | |
|---|---|
| Submission number | 8521640 |
| Application number | EP20169025.2 |
| File No. to be used for priority declarations | EP20169025 |
| Date of receipt | 09 April 2020 |
| Your reference | 17366-EP-EPA |
| Applicant | SOCIETE DES PRODUITS NESTLE S.A. |
| Country | CH |
| Title | BAKED PRODUCT |

| | | |
|---|---|---|
| Documents submitted | package-data.xml | ep-request.xml |
| | application-body.xml | ep-request.pdf (4 p.) |
| | SPECEPO-1.pdf\17366-EP-EPA - 1st filing specification.pdf (36 p.) | SPECEPO-2.pdf\17366-EP-EPA - figures.pdf (6 p.) |
| | OLF-ARCHIVE.zip\17366-EP-EPA - compressed.zip | f1002-1.pdf (1 p.) |

| | |
|---|---|
| Submitted by | CN=secure.epoline.org |
| Method of submission | Online |
| Date and time receipt generated | 09 April 2020, 14:57 (CEST) |
| Message Digest | 12:21:64:FB:1D:C3:C0:D1:64:03:BD:BF:D7:AB:4B:A8:19:69:3F:93 |

**Correction by the EPO of errors in debit instructions filed by eOLF**
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

**DAS access code**
The access code generated for this application and used to retrieve the priority documents from WIPO's Digital Access Service (DAS) is indicated in the document appended to this acknowledgement of receipt. Please note that the appended document is non-public and will not be published.

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

## DAS access code

**To access and retrieve the priority document from WIPO's Digital Access Service (DAS) in respect of**

| | |
|---|---|
| Application number | EP20169025.2 |
| Applicant | SOCIETE DES PRODUITS NESTLE S.A. |

**the European Patent Office has generated the following code:**

| | |
|---|---|
| DAS access code | 03F5 |

**For further information, see OJ EPO 03/2019.**

| | |
|---|---|
| Date and time receipt generated | 09 April 2020, 14:57 (CEST) |

**This unique access code allows the applicant to authorise participating intellectual property offices to retrieve a certified copy of the present application (as priority document) via WIPO DAS.**
**The code will only be valid if the requirements for according a date of filing are met (see OJ EPO 03/2019).**

# 4. CHAPTER IV. Effect of arabinoxylan and wheat bran incorporation on dough rheology and thermal processing of rotary-moulded biscuits

**M. Teresa Molina**, Lisa Lamothe, Deniz Z. Gunes, Sandra M. Vaz & Pedro Bouchon

Being submitted to *Food Chemistry*, 2020.

## 4.1 Introduction

Wheat bran constitutes about 16% of the weight of the wheat grain. It is the main by-product of wheat milling and is mostly used for animal feed (Onipe et al., 2015). It represents about 46% of the total weight of dietary fiber, which mainly consists of arabinoxylans (70%), cellulose (24%) and β-glucans (6%) (Maes & Delcour, 2002). Accordingly, wheat bran is a convenient and inexpensive source of dietary fiber and specifically of insoluble fiber, which has beneficial effects on human health. Said effects include the acceleration of intestinal transit time, prevention/relief of constipation (insoluble fibers), and the reduction of the post-prandial glycaemic response, the serum total, and LDL-cholesterol (soluble fibers) (Bor-denave, Lamothe & Kale, 2020).

Whole wheat flour has been used to enrich baked products with bran fiber, including bread (Lapčíková et al., 2019; Le Bleis et al., 2015), cracker biscuits (Wang et al., 2016; Li et al., 2014) and sugary biscuits (Sozer et al., 2014; Nandeesh et al., 2011; Sudha et al., 2007). Studies show that the quality of the final product is detrimentally affected by bran incorporation because it modifies the rheological properties of the dough. Lapčíková et al. (2019) and Le Bleis et al. (2015) observed that bran addition and small bran particles disrupted the continuous gluten network, affecting dough expansion and producing bread with a low specific volume and high density. Li et al. (2014) showed that bran and especially arabinoxylans limited hydration of gluten proteins, which keeps them from forming appropriate networks for gas retention, precluding dough expansion of saltine crackers during baking and reducing their volume.

Sugary biscuit doughs are quite different from bread or saltine-cracker doughs. Sweet biscuits are formulated with higher amounts of sugar (10-33%, total dough wet basis) and fat (6-21%, total dough wet basis), and with less water (5-10%, total dough weight basis) than bread or saltine-cracker doughs (Manley, 2011). During sweet-biscuit making, the mixing process is commonly divided into two stages to reduce the contact between flour and water as much as possible and thus limit the formation of a gluten network before baking (Pareyt & Delcour, 2008a). Although the gluten network is limited in these products, bran enrichment may have a considerable effect on the rheological properties and baking performance of the biscuit dough, thus impacting final biscuits attributes. Milling of bran has been applied as a pre-processing step before its incorporation into whole grain products because large bran particles are highly visible and substantially affect their appearance (Bordenave et al., 2020). Sozer et al. (2014) examined the effect of bran particle size and bran supplementation from 0 to 30% total weight (based on flour replacement) during processing of rotary-moulded biscuits. They showed that the incorporation of fine bran particles (68 µm, mean diameter) increased the hardness of biscuits and the degree of gelatinized starch after baking, while coarser bran (450 µm, mean diameter) did not produce a significant effect. Likewise, Sudha et al. (2007) analysed the effect of bran enrichment (particle size lower than 150 µm) from 0 to 40% total weight (based on flour replacement) on some physicochemical properties of rotary-moulded biscuits. They noticed that biscuits became harder to break as wheat bran content increased, but no further explanation was provided.

As mentioned above, arabinoxylans are the most important non-starch polysaccharides found in whole wheat flour and refined flour, and they have the highest water holding capacity among the flour polymers. Native starch, damaged starch and gluten proteins can hold 0.30-0.45, 1.0-10.0 and ~2.8 grams of water per gram of dry matter, respectively, while arabinoxylans can hold 4-10 grams of water per gram of dry matter (Kweon et al., 2011; Van Craeyveld, 2009). Most of the scientific knowledge about the effect of arabinoxylans on biscuits has been taken from correlations between biscuit dimensions and the solvent retention capacity (SRC) test (Duyvejonck et al., 2011; Colombo et al., 2008; Ram & Singh, 2004). The SRC is a solvation test for flours that can be used to study the functional contribution of glutenins, damaged starch and arabinoxylans to the flour-swelling behaviour

(Kweon et al., 2011). Ram et al. (2004) analysed the relationship between the SRC values of fifty Indian wheat cultivars and the sugar-snap quality, and detected a strong negative correlation ($r = -0.78$) between the SRC test (for arabinoxylans) and the diameter of sugar-snap biscuits, suggesting that the water absorption of arabinoxylans detrimentally affected the biscuit quality. By contrast, Duyvejonck et al. (2011) examined the contribution of flour constituents to the SRC profile for nineteen European wheat flours and did not observe any relationship between SRC values and total, water-extractable or water-unextractable arabinoxylans. Regarding the isolated effect of arabinoxylans in biscuits, Pareyt et al. (2011) analysed the replacement of flour or sugar by arabinoxylan oligosaccharides (enzymatically derived from wheat bran arabinoxylan) on the quality attributes of sugar-snap biscuits. They found that flour replacement by arabinoxylan oligosaccharides produced unacceptable biscuits, whereas sucrose replacement by arabinoxylans oligosaccharides (up to 30 g per 100 g of sucrose) resulted in biscuits that were similar in diameter and height to control biscuits, suggesting their potential role as a sucrose replacer and dietary fiber contributor. To better understand these phenomena, it would be of great interest to examine the contribution of arabinoxylans compared to the direct addition of wheat bran in the rheological behaviour and the structure of sweet biscuits during baking.

Accordingly, the aim of this study was to analyse the effect of water-unextractable and water-extractable wheat arabinoxylans addition and the effect of wheat bran flour enrichment on the dough rheology and starch gelatinization and on the resultant microstructure of rotary-moulded biscuits.

## 4.2 Materials and Methods

### 4.2.1 Materials

Soft wheat was supplied by Molinera San Cristobal (Santiago, Chile). Soluble wheat arabinoxylan (P-WAXYM, lot 40601) and insoluble wheat arabinoxylan (P-WAXYI, lot 120801b) were purchased from Megazyme Ltd. (Bray, Ireland). Fat was kindly provided by

Team Foods Spa (Santiago, Chile). Granulated sucrose was obtained from IANSA (Santiago, Chile), soy lecithin from Cargill (Santiago, Chile), ammonium bicarbonate from Pistor AG (Jura-Nord Vaudois, Switzerland), sodium bicarbonate from Andimex S.A. (Santiago, Chile), monocalcium phosphate from Blumos (Santiago, Chile), and salt from K+S Chile S.A. (Santiago, Chile).

Soft wheat was subjected to a milling process using a RT-1 pill mill (FP Spomax SA, Poland) to obtain refined flour (RF) and the wheat bran large fraction (72% of particles > 500 µm, with a Sauter diameter = 790 µm). The flour extraction rate was 75.7%. Part of the wheat bran was subjected to additional milling using a Mikro-Pulverizer MP1 (Micron Powder Systems, USA) to reduce its particle size and obtain the wheat bran small fraction (4% of particles > 500 µm, with a Sauter diameter = 307 µm).

### 4.2.2 Preparation of flour blends

Whole flours with different bran particle sizes were reconstituted based on the flour extraction rate (75.7%). The large or small bran fraction was used to obtain whole flour with large bran (WFL) and whole flour with small bran (WFS), respectively (The chemical composition of the different flours is presented in **Table 4.1**). In order to analyse the effect of whole flour enrichment on biscuit-making, different flour mixes were prepared by replacing 25, 50, 75 or 100% of the refined flour with WFL or WFS. The flour blends were dry blended using a N50 Hobart mixer with a flat beater at a low speed (60 rpm) to homogenize the mixture of refined and whole flours.

In order to understand the influence of arabinoxylans during biscuit-making by isolating them from the other components of bran, a second system was examined which consisted of blends of refined flour enriched with water-unextractable (WUAX) and water-extractable (WEAX) arabinoxylans (from Megazyme) only. Four *model flours* were prepared by adding the amount of WUAX and WEAX necessary to achieve specific arabinoxylans required for flours with either 50% or 100% WFS or WFL replacement. These model flours were referred as 50MFS, 100MFS, 50MFL, and 100MFL, respectively.

**Table 4.1** The chemical composition (% of total weight) of refined flour (RF), whole flour with large bran fraction (WFL), and whole flour with small bran fraction (WFS).

| Flour components | RF | WFL | WFS |
|---|---|---|---|
| Starch | 70.6 | 58.5 | 59.2 |
| Moisture | 13.9 | 13.2 | 12.1 |
| Proteins | 10.0 | 11.4 | 11.5 |
| Total fiber | 3.6 | 13.0 | 13.2 |
| Lipids | 1.3 | 2.0 | 2.1 |
| Ash | 0.6 | 1.9 | 1.9 |

### 4.2.3 Proximate composition analysis

The chemical composition of the flour was measured using the following analyses for starch (AACC 76-13.01), moisture (AACC 44-15.02), proteins (AACC 46-10.01), fiber (AOAC 985.29), and lipids (AOAC 922.06).

The arabinoxylan content of flours was determined using the phloroglucinol colorimetric method, as described by Ramseyer et al. (2011). To determine the total arabinoxylan (TAX) content, 125 mg of flour was hydrated with 25 ml of distilled water in a 50 ml conical centrifuge tube. The sample was suspended by vortexing for 10 s. Then, 1 ml aliquot was immediately removed from the suspension and transferred into a reaction tube (30 g borosilicate glass, Anton Paar GmbH) using a 5-ml pipette tip. The total volume was brought to 2 ml by adding distilled water. To determine the water-extractable arabinoxylan (WEAX) content, 125 mg of flour was suspended with 25 ml of distilled water by vortexing. The suspension was placed in an orbital shaker (3D mini-shaker, Boeco Germany) for 30 min at room temperature (23°C). Afterwards, it was centrifuged at 2500× g for 10 min, and 1 ml supernatant aliquot was transferred into a reaction tube. The reaction agent was prepared by mixing 110 mL of glacial acetic acid, 2 mL of concentrated hydrochloric acid, 5 mL of 20% w/v phloroglucinol in absolute ethanol, and 1 mL of 1.75% w/v glucose. Then, 10 ml of the reaction agent was added to each reaction tube containing the samples for TAX and WEAX measurements. The tubes were placed in a boiling water bath for 25 min followed by rapid cooling in a water bath with ice up to 23°C, and they were externally covered using aluminium foil. The absorbance of the sample was read at 552 and 510 nm, and these values were subtracted to remove the influence of hexose sugars. This was done immediately after cooling, as the absorbance may decrease after 10 min (Kizsonas et al., 2012). A standard curve was obtained using D-(+)-Xylose (Sigma-Aldrich). A solution of 100 mg of xylose was prepared in 100 ml of distilled water (solution A). Aliquots of 0.02, 0.05, 0.1, 0.2, 0.3, and 0.4 ml were taken from solution A, and the volume was brought up to 2.0 ml with distilled water. Thus, standard solutions of 0.01, 0.025, 0.05, 0.1, 0.15, and 0.2 mg of xylose per ml of water were obtained. These standard solutions were subjected to the same procedure as flours (in triplicate). The arabinoxylan content was calculated using the xylose standard curve ($y = 3.4895x, R^2 = 0.992$) and it was expressed as mg xylose equivalent. The water-unextractable arabinoxylan (WUAX) was obtained as the difference between TAX and WEAX.

### 4.2.4 Water retention capacity of flours

Water retention capacity (WRC) was measured in triplicate using the procedure described by Hemdane et al. (2018). Flour (1.0 g) was suspended with 10 ml of distilled water in a 50-ml conical centrifuge tube by vortexing for 20 s. The sample was soaked for 60 min at room temperature (25°C), followed by centrifugation at 4000× g for 10 min. The supernatant was carefully discarded, and the pellet was drained for 15 min. Finally, the pellet in the tube was weighed, and the WRC was quantified by subtracting the initial mass of the sample and tube. The results were expressed as g water retained per g dry sample.

### 4.2.5 Biscuit dough preparation

Control dough was formulated by mixing RF (65.2%, d.b.), sucrose (19.1%, d.b.), fat (13.1%, d.b.), leavening agents (2.2%, d.b.), soy lecithin (0.3%, d.b.), and salt (0.1%, d.b.) with water (11%, d.b.). The amount of added water was adjusted slightly in each formulation to allow adequate dough formation. However, the proportion of all ingredients on a dry basis (d.b.) remained constant.

The biscuit dough was prepared in two steps: the creaming phase and the dough phase. During the first step, sucrose, fat, water, soy lecithin, salt, sodium bicarbonate, and ammonium bicarbonate were mixed for 5 min in a N50 Hobart mixer using a flat beater at medium speed (124 rpm) as part of the creaming phase. Thereafter, the flour and monocalcium phosphate were added, and the mix was blended for 2 min at low speed (60 rpm) to obtain the biscuit dough. The dough was moulded in rectangular molds (53 mm × 34 mm × 3 mm) and then baked at 170°C for 13 min until a final moisture content of 2.3 ± 0.3% (w.b.) was reached.

### 4.2.6 Rheological characterization

Dynamic oscillatory rheological measurements of biscuit doughs were conducted on a Physica MCR 501 rheometer (Anton Paar GmbH, Austria) following Ahmed et al. (2013) with some modifications. The geometry of the measuring system was serrated parallel plates with 50 mm plate diameter. Before measurements were taken, the moulded dough was allowed to rest for 20 min for relaxation. Strain sweep tests (at 25°C) were firstly conducted at a frequency of 1 Hz over 0.001-0.1% strain range to ensure that all other measurements were within the linear viscoelastic regime. Then, frequency sweep tests (at 25°C) were performed in duplicate (2 batches per condition) from 0.01 to 10 Hz at a strain of 0.005% to determine the elastic modulus (G'), the loss modulus (G''), and the loss tangent ($\tan\delta = G''/G'$) as a function of frequency.

### 4.2.7 Texture measurements

The firmness of the biscuit doughs was determined using a penetration test in a TA.HD*plus*C Texture Analyzer (5 kg loadcell, Stable Microsystems, United Kingdom), following the procedure described by Colakoglu & Özkaya (2012). The dough sample (~100 g) was weighed in a dough preparation set (A/DP) to remove the randomly distributed air inside the sample and to get a flat surface before the penetration test. The measurements were carried out using a P/6 cylindrical probe of 6 mm diameter. The probe penetrated the dough 20 mm at a test speed of 3 mm/s. Six replicates were taken of each batch, and this procedure was completed twice (2 batches per condition). The positive force value (N) at the maximum penetration depth was taken as a texture parameter of the dough firmness.

The hardness and fracturability of the biscuits was measured two days after they were made using a TA.XT Texture Analyzer (5 kg loadcell, Stable Microsystems, United Kingdom). Twenty biscuits per batch (3 batches per condition) were analysed using a three-point bending test (HDP/3PB probe) with a support span of 36 mm and test speed of 1 mm/s over a distance of 5 mm. The maximum breaking force (N) upon compression was used as a texture descriptor of hardness in biscuits, and the distance (mm) to the breaking point was used to represent fracturability (Jambrec et al., 2013).

### 4.2.8 Differential Scanning Calorimetry (DSC)

DSC analysis was performed in triplicate using a Mettler Toledo DSC823e (METTLER TOLEDO, USA) in order to quantify the degree of starch gelatinization (DG) in biscuits after baking following Molina et al. (2020). Rotary-moulded dough was dried at 43°C for 20 h so that it could be ground because crumbles of dough are difficult to hydrate with water and erroneous curves might be obtained. The ground sample (~16 mg, raw dough or baked biscuit) was placed in a 160 µL aluminium pan with distilled water ensuring a 1:4 ratio between the sample and the water. A pan containing distilled water (the same amount used for the sample preparation) was used as a reference. The pans were hermetically sealed and equilibrated at room temperature for 18 h prior to the analysis. The samples were heated from 25 to 90°C at 5°C/min, and the degree of starch gelatinization (DG, %) was quantified using the enthalpy values of raw ($\Delta H_{dough}, J/g$) and baked dough ($\Delta H_{biscuit},\ J/g$), according to **Eq. (4.1)**:

$$DG(\%) = \left(\frac{\Delta H_{dough} - \Delta H_{biscuit}}{\Delta H_{dough}}\right) \cdot 100 \quad (4.1)$$

where ($\Delta H_{dough}$) corresponds to the enthalpy of all of the starch granules embedded in the dough, which were forced to gelatinize in excess water, and ($\Delta H_{biscuit}$) corresponds to the enthalpy of the starch granules that did not complete the gelatinization process during baking. Therefore, $\Delta H_{dough} - \Delta H_{biscuit}$ denotes the enthalpy of the gelatinized starch during baking. The enthalpy was expressed as J/g of dry starch in order to standardize the enthalpy values with respect to the amount of starch in the flour.

### 4.2.9 Microstructural analysis

#### *Scanning Electron Microscopy (SEM)*

Rotary-moulded biscuits were defatted prior to SEM analysis in order to reduce the fat interference during the visualization as suggested by Pareyt et al. (2010a). The defatting procedure was carried out by soaking them in hexane (50 ml) for 30 min at room temperature. This process was repeated seven times and the solvent was replaced each time. The defatted biscuits were subsequently placed on a metallic stub equipped with double-side conductive carbon tape and tightened on stub with a silver conductive adhesive paste and a copper/nickel conductive tape. Before imaging, the biscuits were coated with a 10 nm gold layer using a SCD500 sputter coater (Leica Microsystems, Switzerland). Finally, the biscuits were examined using a Quanta F200 Scanning Electron Microscope (FEI Company, the Netherlands) in low vacuum mode at 0.2 mbar by collecting secondary electrons with Everhart-Thornley Detector (ETD) and Back Scattered Electrons (BSE), at an accelerating potential of 5 kV with magnifications ranging from 200× to 1500×.

#### *X-ray micro-computed tomography (X-ray micro-CT)*

The microstructure of rotary-moulded biscuits was characterized on a Skyscan 1272 X-ray computed microtomography system (version 1.1.17, Bruker Corp., Belgium). The image acquisition was carried out with an X-ray source operated at a voltage of 40 kV and a current of 250µA using an exposure time of 800s per frame over an interval of 0-180° with a rotation step of 0.2° and two frames averaging on each rotation step. Three biscuits were scanned for each condition.

Around 962 projection images were obtained from the image acquisition, and they were reconstructed using an adapted cone-beam filtered back projection algorithm (Feldkamp et al., 1984) and reconstruction software (NRecon v. 1.7.3, Bruker Corp., Belgium). 2-D cross-sectional images were obtained with a resolution of 2016 × 1344 pixels and a voxel size of 5.5µm × 5.5µm × 5.5µm. During the reconstruction step, critical parameters were set to obtain good quality reconstructed images: *thermal correction* (X/Y alignment with a reference scan), *misalignment compensation* (post-alignment), *smoothing* (1, using Gaussian Kernel = 2), *ring artifacts reduction* (= 8), and *beam-hardening correction* (= 40%).

### 4.2.10 Statistical analysis

The experimental data were acquired in triplicate (3 batches per formulation) and analysed with R, version 3.6.1. For the statistical analysis, the bootstrap method introduced by Efron (1992) was used as it makes no assumption about the underlying population distribution (*i.e.* a non-parametric approach). Accordingly, the analysis performed in this study did not assume that data come from a normal distribution and instead, fully accepted that the population distribution was unknown. The experimental data were used as an initial database to perform the analysis. Next, one thousand random replicates were extracted with replacement from the initial database to estimate the unknown population distribution of the statistical parameters of interest (*mean* in this case) and confidence intervals. Finally, 95% confidence intervals were used to test differences between the means of the different dough formulations.

## 4.3 Results and Discussion

### 4.3.1 Arabinoxylan content and water retention capacity of flours

The water-extractable (WEAX) and total arabinoxylans (TAX) fractions were determined in refined and whole flours (RF, WFL, and WFS). The TAX and WEAX contents were 2.20 ± 0.21% and 0.49 ± 0.05% in refined flour, 5.84 ± 0.44% and 0.51 ± 0.07% in whole flour with large bran, and 5.09 ± 0.25% and 0.55 ± 0.07% in whole flour with small bran, respectively. The WUAX fraction, an important structural component in the cell wall of the endosperm and bran (Dornez et al., 2011), constituted the greatest proportion of arabinoxylans. These results align with those of Kiszonas et al. (2012) and Saulnier et al. (2007), who reported that whole flour contains about 3.5-5.9% TAX and 0.3-0.6% WEAX, whereas refined flour contains about 2.2% TAX, and approximately 0.5% WEAX.

**Table 4.2** shows the water retention capacity determined in refined flour (RF), whole flours (WFL and WFS), and model flours prepared with arabinoxylans (MFL and MFS). Overall, the water retention capacity increased as refined flour was enriched with whole flour or wheat arabinoxylans. A significant increase (p-value < 0.05) was observed in the water retained by flour blends enriched with whole flour in particular at a replacement equal or over 50%. Bran particle size marginally affected this value; blends with small bran particles retained ~2% more water than blends with large bran, but differences were not always significant. The water retention capacity in model flours prepared with arabinoxylans was significantly higher (p-value < 0.05) than in blends enriched with whole flour (with an equivalent arabinoxylans content). This shows that isolated fiber can retain more water than when it is inside of the bran matrix. The WUAX/WEAX ratio in model flours increased from 3.5 (refined flour) to 7.5 and 6.3 in 50MFL and 50MFS, and to 10.9 and 8.7 in 100MFL and 100MFS, respectively. This suggests that both WUAX and WEAX, but especially WUAX, may influence the increase in flours' water-retention capacity due to their strong tendency to absorb water, as reported by Courtin & Delcour (2002). The water-retention ability of wheat bran and specifically of arabinoxylans has been attributed to their structure, either through interaction with hydroxyl groups or through capillary forces due to the existence of micropores (Boita et al., 2016; Jacobs et al., 2015), as will be discussed next.

**Table 4.2** Water retention capacity (expressed as g H2O/g dry sample × 100) measured in the refined flour (RF), in the blends enriched with whole flour (WFL or WFS), and in model flours enriched with arabinoxylans (MFL or MFS). Data are observed means ± confidence intervals at 95%. Different scripts denote significant differences ($p < 0.05$).

| Enrichment with Whole Flour | Flour nomenclature | Water retention capacity | C.I. at 95% |
|---|---|---|---|
| 0% | RF - Control | 64.1 [a] | (60.6, 66.1) |
| 25% | WFL | 65.3 [a] | (63.4, 66.5) |
| | WFS | 66.4 [a] | (66.4, 66.5) |
| 50% | WFL | 69.6 [b] | (68.2, 70.7) |
| | WFS | 71.3 [c] | (70.9, 71.4) |
| 75% | WFL | 74.4 [d] | (71.4, 76.0) |
| | WFS | 75.4 [d] | (73.5, 77.0) |
| 100% | WFL | 79.4 [e] | (77.7, 82.0) |
| | WFS | 81.9 [f] | (80.8, 83.0) |
| **Enrichment with Arabinoxylans** | **Flour nomenclature** | **Water retention capacity** | **C.I. at 95%** |
| 50% | MFL | 73.7 [d] | (72.6, 74.3) |
| | MFS | 72.2 [bcd] | (70.4, 75.9) |
| 100% | MFL | 92.7 [h] | (90.8, 95.4) |
| | MFS | 86.5 [g] | (86.2, 87.1) |

### 4.3.2 Firmness and rheological analyses of biscuit dough enriched with wheat fiber

The viscoelastic properties of dough may influence the moulding stage and baking performance, impacting the final attributes of rotary-moulded biscuits. As such, firmness and rheological parameters were measured in fiber-enriched biscuit doughs. **Figure 4.1** shows the firmness of doughs enriched with whole flour containing large or small bran (**Figure 4.1.A**) as well as those enriched with arabinoxylans (**Figure 4.1.B**). The incorporation of whole flour significantly increased (p-value < 0.05) dough firmness compared to dough made with refined flour, which aligns with results reported by Nandeesh et al. (2011) for rotary-moulded biscuits and by Filipčev et al. (2017) for sugar-snap and biscuits.

Additionally, the bran particle size significantly affected (p-value < 0.05) this value. The firmness was ~8% higher in dough with small bran particles compared to the dough with large bran at a replacement of 25% and rose to ~17% at 50 and 75% substitution levels. The highest values of firmness were obtained in whole flour (*i.e.* 100% whole flour replacement), while dough containing finer bran had the greatest firmness. These results suggest that bran particles, especially smaller ones, produce a physical hindrance along the dough structure, as previously discussed by Sozer et al. (2014) regarding sugar-snap dough and Wang et al. (2016) regarding cracker dough. For their part, arabinoxylans (WEAX and WUAX) had a lower impact on dough firmness. This parameter was more affected when doughs were prepared with model flours simulating 100% replacement. Given that the effect of bran particle size is removed in these flours, the firmness response is mainly influenced by the WUAX/WEAX ratio. This ratio was higher in dough with MFL than in dough with MFS, so the increased firmness may be explained by the higher water retention capacity of WUAX.

A rheological analysis was performed to arrive at a better understanding about the effect of whole flour or arabinoxylans incorporation on the viscoelastic response of rotary-moulded dough. **Figure 4.2** shows the frequency sweep ($G'/G'_{control}$) and loss tangent ($G''/G'$) curves of doughs enriched with whole flours (**Figure 4.2.A and 4.2.B**) and arabinoxylans (**Figure 4.2.C**). The storage modulus of each formulation was divided by the storage modulus of the dough prepared with refined flour (control) to better distinguish the effect of the enrichment on this variable. Overall, dough samples showed a predominant solid-like behaviour at the frequency range of 0.1 to 10 Hz, since the storage modulus (G') was always higher than the loss modulus (G''). This is consistent with the findings published by Li et al. (2019) and Li et al. (2014), who also observed an elastic-like behaviour ($G' > G''$) in doughs enriched with wheat bran. Furthermore, the elastic component of doughs gradually increased as they were supplemented with wheat fiber, but the values differed among samples containing different bran particle sizes or arabinoxylans. A rather similar elastic response was observed in doughs prepared with whole flours at 25% replacement (**Figure 4.2.A and 4.2.B**), with either large or small bran particles. However, over 50% replacement, ($G'/G'_{control}$) increased much more in doughs with small bran particles. Similar behaviour was observed in doughs enriched with arabinoxylans (**Figure 4.2.C**), where the amount of AX increased the elastic response and the WUAX/WEAX ratio increased with respect to refined flour, as explained in the previous section. With respect to the loss tangent ($G''/G'$), this value was significantly lower (p-value

< 0.05) in doughs containing finer bran particles, and it decreased even more when arabinoxylans were added, probably due to their higher water retention capacity.

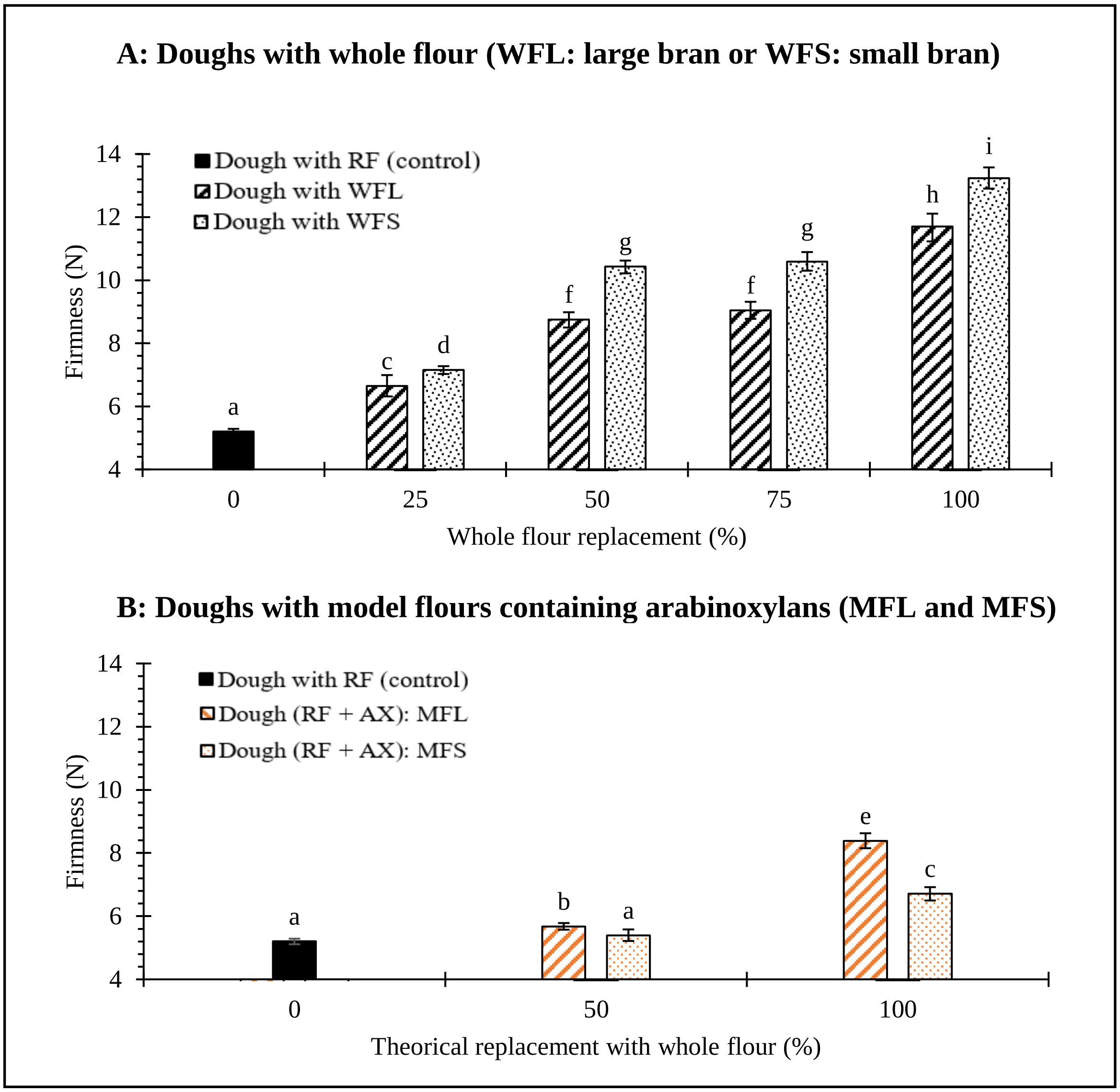


**Figure 4.1** Firmness (N) of control dough, doughs prepared by replacing 25, 50, 75 or 100% of the refined flour with whole flour with large bran (FWL) and whole flour with small bran (WFS) [A], and model flours enriched with arabinoxylans with an equivalent content to that found in flours with 50 or 100% whole flour replacement (MFL: large bran; MFS: small bran) [B]. Data are observed mean ± confidence intervals at 95%. Different superscript letters refer to significant difference ($p < 0.05$).

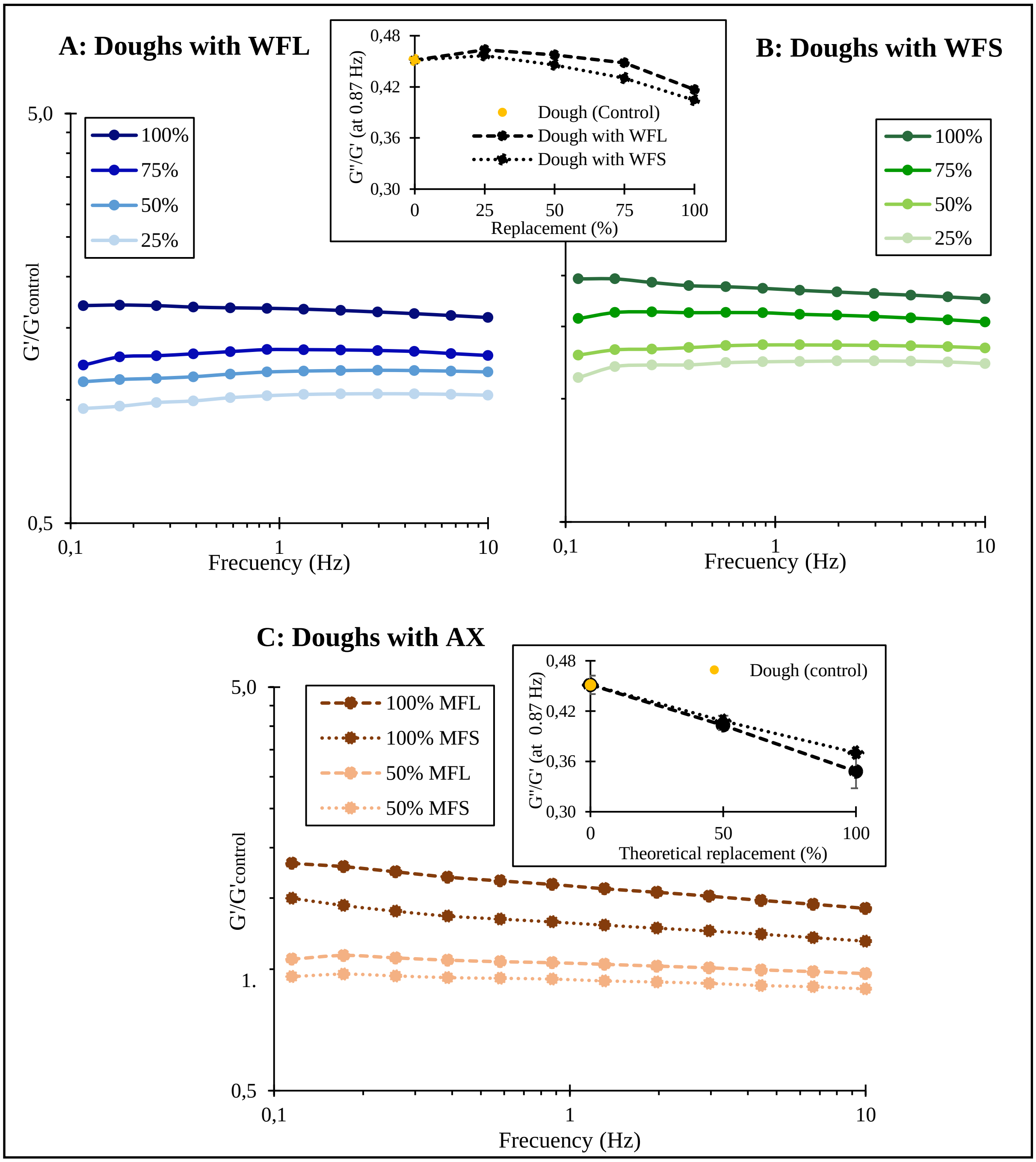


**Figure 4.2** Frequency sweep curves represented by G'/ $G'_{control}$ (where the *control* corresponds to the dough prepared with refined flour) as a function of frequency (Hz) of doughs prepared by replacing 20, 50, 75 or 100% of the refined flour with whole flour with large bran (WFL) [A] and whole flour with small bran (WFS) [B], and model flours enriched with arabinoxylans [C]. The loss tangent curves (G''/ G') as a function of replacement at a frequency of 0.873 Hz are also shown.

### 4.3.3 Starch gelatinization during baking and microstructure of fiber-enriched biscuits

In order to understand the influence of whole flour and arabinoxylans on starch gelatinization, the degree of gelatinized starch (DG) of rotary-moulded biscuits was quantified. Results were determined according to **Eq. (4.1)** and are presented in **Figure 4.3**. Overall, partial gelatinization (< 38%) was observed for all biscuits, but the DG varied according to the enrichment with arabinoxylans or whole flour. In biscuits prepared with refined flour (control), only ~24.3% of native starch gelatinized during baking. This value rose significantly as large bran concentration increased, up to 36.3%, as shown in **Figure 4.3.A (WFL curve**). However, no significant differences were observed in samples prepared with whole flour with small bran particles compared to refined flour except from a minor significant increase (3%) at 75% replacement (**Figure 4.3.A – WFS curve**). The increase in starch gelatinization related to bran inclusion may be due to the high water-retention capacity of bran, which can slowly release and make water available as cooking progresses (Hemdane et al., 2018; Roozendaal et al., 2012). In fact,  Roozendaal et al. (2012) concluded that bran can take up 200-500 times its own body mass in absorbed water (inside its micro-capillaries) and can release large amounts of water during baking. By contrast, Sabanis et al. (2009) suggested that fiber water retention may limit starch gelatinization. However, these authors did not consider the effect of the decrease in starch content due to wheat bran replacement when reporting the gelatinization enthalpy of dough enriched with wheat bran. This reduction is expected to diminish the gelatinization enthalpy as less starch is available in the system, affecting the reported results. In this study, the degree of gelatinization was quantified measuring the enthalpies of the dough and the baked biscuit prepared with the same formulation. As such, all values were standardized according to the amount of dry starch.

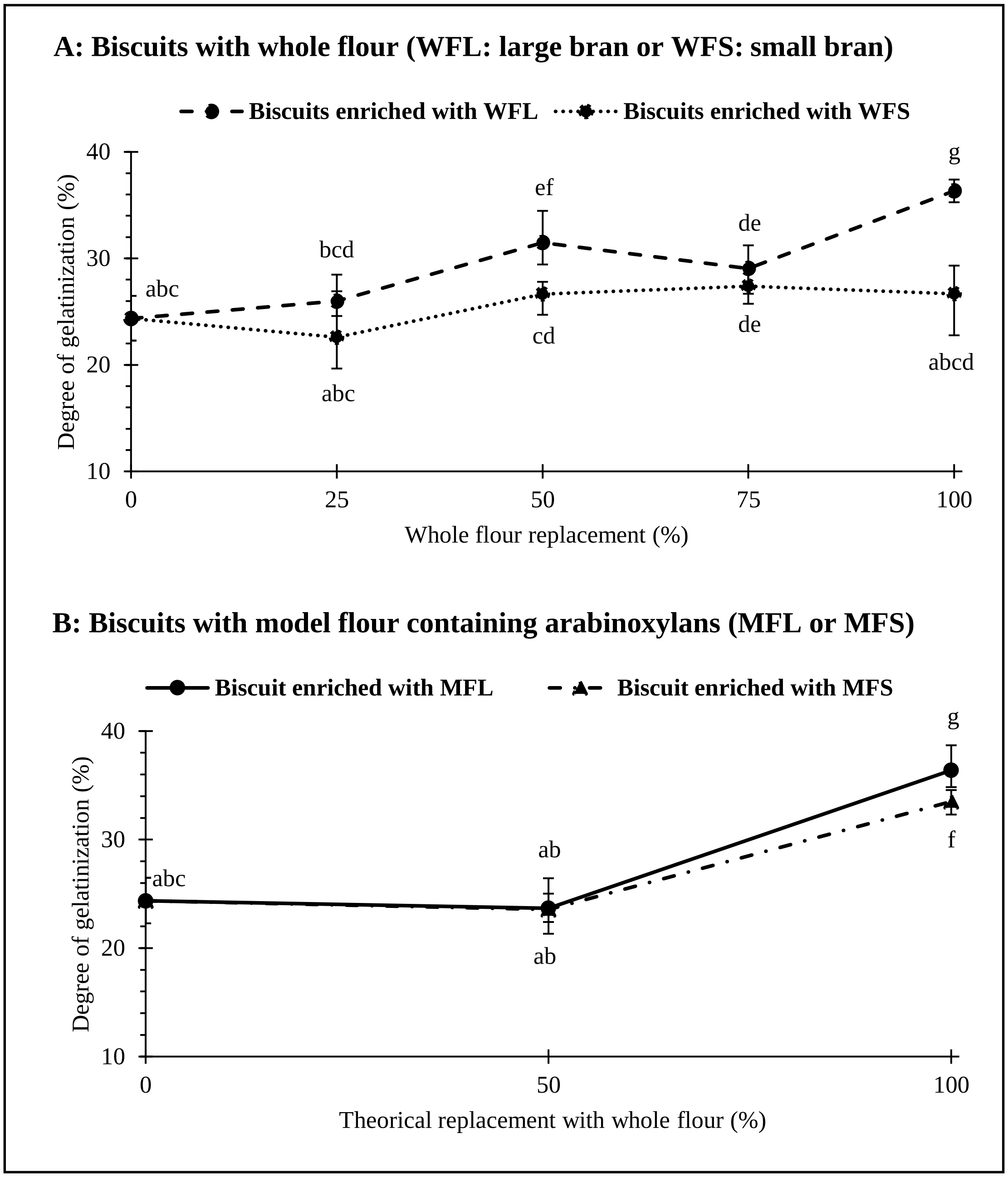


**Figure 4.3** Degree of starch gelatinization (%) in rotary-moulded biscuits produced with refined flour, with doughs prepared replacing 25, 50, 75 or 100% of the refined flour with whole flour with larg bran (WFL) and with whole flour with small bran (WFS) [A], and with model flours enriched with arabinoxylans with an equivalent content to that found in flours with 50 or 100% whole flour replacement (MFL: large bran; MFS: small bran) [B]. Data are observed mean ± confidence intervals at 95%. Different script letters refer to significant difference ($p < 0.05$).

The difference in starch gelatinization due to bran particle size (**Figure 4.3.A**) suggests that the microstructure of fibers may play an important role. To better examine this possibility, microstructural observations were performed using scanning electron microscopy (SEM) and X-ray micro-computed tomography (X-ray micro-CT), as shown in **Figure 4.4**. **Figure 4.4.a** shows that the large bran fraction contains clear micropore arrangements, which are not observed when examining the small bran fraction (**Figure 4.4.b),** probably due to its destruction during extended milling, as previously exposed by Holopainen-Mantila & Raulio (2016). This micro-arrangement is also observed in rotary-moulded biscuits prepared with whole flour with large bran particles (**Figure 4.4.f**), and it is not detected in the control biscuit (**Figures 4.4.e**) or in biscuits prepared with WFS (**Figures 4.4.g**). X-ray micro-CT images (**Figures 4.4.j and 4.4.k)** show some pericarp fragments (highlighted in light yellow) with their characteristic micropores surrounding the air pores, in accordance to previous observations from Hemdane et al. (2018), using SEM.

These observations support the hypothesis that water could be held inside of these capillaries and then released in a controlled manner during baking. As explained by Jacobs et al. (2015), bran capillaries may play a key role in enclosing water at micro- and nano-scales, mainly during the first 2.5 min of unconstrained absorption as long as the bran is not subjected to external forces. They also mentioned that the higher hydration properties of coarse wheat bran are attributable to weakly bound water located inside the micropores or between particles due to a less efficient stacking compared to fine bran particles. In the case of biscuits, the weakly bound portion of water may be removed from the large bran matrix during mixing, as the dough is constantly subjected to mechanical forces, and only the strongly bound water (inside the bran nanopores or through hydrogen bonding) may remain retained. However, after moulding, each moulded dough remains still on the baking tray for a few minutes (5 to 7 min, depending on the moulding rate) before the baking phase. It is suggested that this resting time may be enough for the coarse bran particles to rehydrate by reabsorbing the water in their micropores, while small bran particles are not able to reabsorb water due to the lack of micropores. As heating takes place during baking, the water inside of the capillaries of coarse bran could be slowly released, allowing starch granules to partially undergo a thermal transition as cooking progresses.

Arabinoxylans' direct inclusion also modified the degree of starch gelatinization (**Figure 4.3.B**). Although DG was unaffected (p-value > 0.05) in biscuits prepared with 50MFS and 50MFL (mean DG of 23.6 or 23.7%), this value increased up to ~33.5 and ~36.3% when biscuits were formulated with 100MFS and 100MFL, respectively. These results show that the amount of arabinoxylans (WUAX and WEAX) affected the degree of starch gelatinization as well as the increase in the WUAX/WEAX ratio, as discussed in previous sections. Additionally, the DG of these biscuits (100MFL and 100MFS) was similar to the DG obtained in biscuits enriched with 100% of whole flour with large bran. With regard to the microstructure, **Figure 4.4.c** shows that water-extractable arabinoxylans (WEAX) appear as an agglomeration of nanoparticles, whereas **Figure 4.4.d** displays water-unextractable arabinoxylans (WUAX) which contain micropore arrangements similar to those found in the large fraction of wheat bran. Furthermore, this microporous structure was also observed in the biscuit prepared with 100MFS or 100MFL, as shown in **Figures 4.4.h and 4.4.i**. As suggested for biscuits prepared with large bran, the porous structure of WUAX may explain the higher percentage of gelatinized starch in biscuits prepared with 100MFL or 100MFS as a result of the higher WUAX/WEAX ratio in these samples. Certainly, further research is needed to better understand this phenomenon.

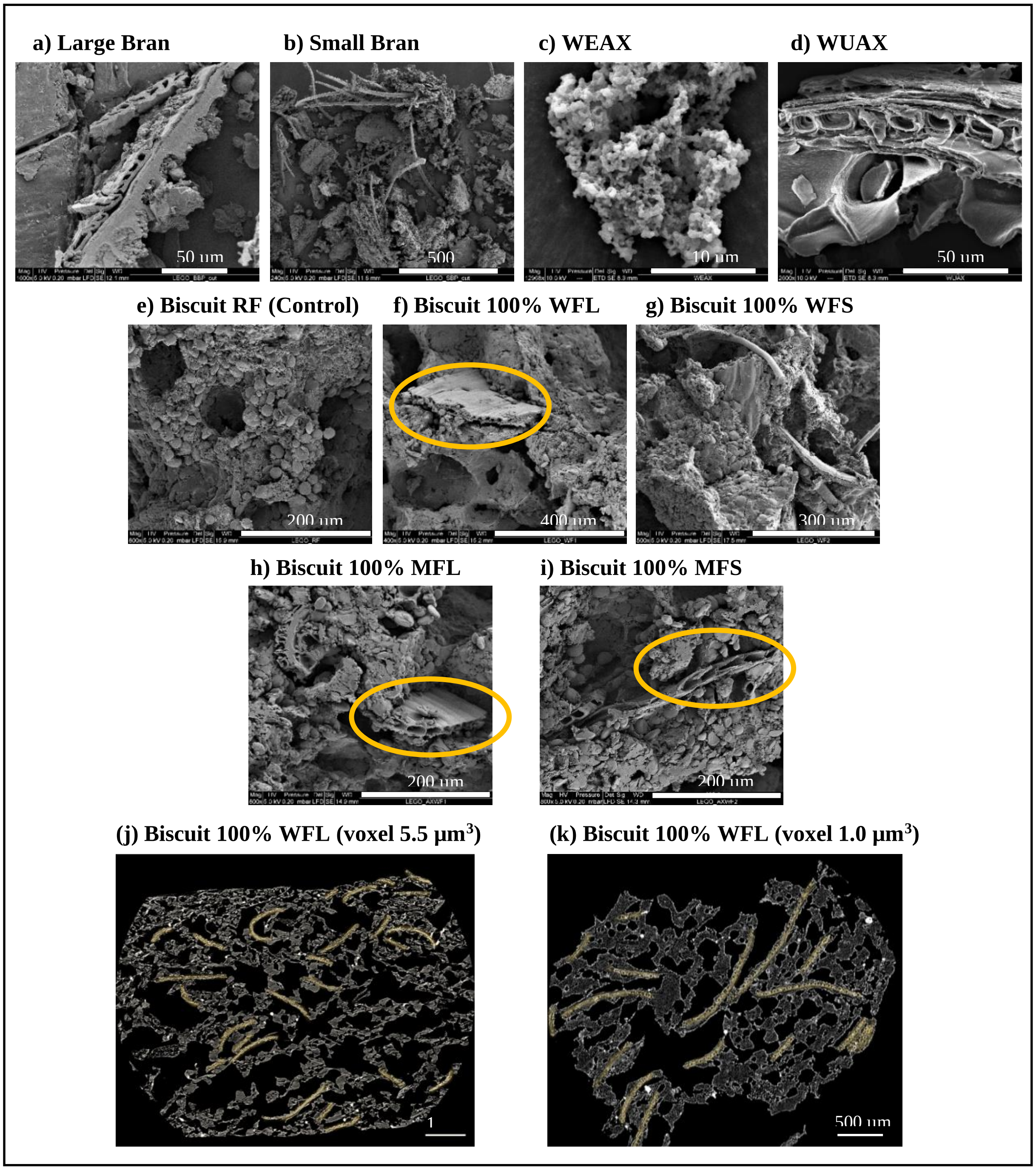


**Figure 4.4** Scanning electron microphotographs (at magnifications between 240× and 12968×) of the following fibers: large bran (a), small bran (b), water-extractable arabinoxylan (c), and water-unextractable arabinoxylan (d). Also, cross-sectional scanning electron microphotographs (at magnifications between 400× and 800×) of biscuits prepared with refined flour (e), whole flour and large bran (f), whole flour and small bran (g), and model flour with an equivalent arabinoxylans content to that found in flours with 100% whole flour replacement (MFL or MFS) (h and i). Images (j) and (k) are cross-sectional image (z plane) obtained by X-ray µCT of a biscuit prepared with 100% whole flour with large bran fraction (WFL), which is highlighted in light yellow.

SEM and X-ray micro-CT images showed that bran fractions were distributed along the biscuit matrix. This aspect may influence the final texture. Additionally, differences in dough rheology and thermal transition due to fiber enrichment were mainly observed at 50% whole flour replacement and above. To better understand this aspect on the texture of rotary-moulded biscuits, the hardness and the fracturability of biscuits prepared with 50 or 100% whole flour replacement were measured, as shown in **Figure 4.5**. The maximum breaking force in the bending test, which is reached upon breakage, is usually used as a texture descriptor of hardness in biscuits, and a higher peak force means a harder biscuit (Canalis et al., 2018). As shown in **Figure 4.5.A**, hardness significantly increased when biscuits were enriched with whole flour, and biscuits with 100% replacement had the highest values. This aligns with the findings of Sudha et al. (2007) and Jribi et al. (2020), who showed that the incorporation of wheat bran increased the breaking strength of rotary-moulded and sugar snap biscuits, respectively. Bran particle size only affected the hardness of biscuits at 50% replacement, but only 1 N difference was observed when comparing small and large bran enrichment.

Enrichment with whole flour and bran particle size significantly affected ($p$-value < 0.05) the fracturability of biscuits, as shown in **Figure 4.5.B**. This parameter is obtained from the force-distance curve and corresponds to the distance to the breaking point, where a lower value is related to a biscuit that is less compressible and more breakable (Jambrec et al., 2013). Accordingly, biscuits with small bran particles had slightly lower fracturability than biscuits prepared with refined flour, as the distance to the breaking point increased from 0.25 mm (biscuit RF) to 0.30 mm (biscuit enriched with WFS). Biscuits enriched with whole flour and large bran had the lowest values of fracturability. Overall, these results indicate that a more compressible and less fracturable structure may be obtained when biscuits are enriched with bran, but the effect would be more pronounced when large bran particles are used.

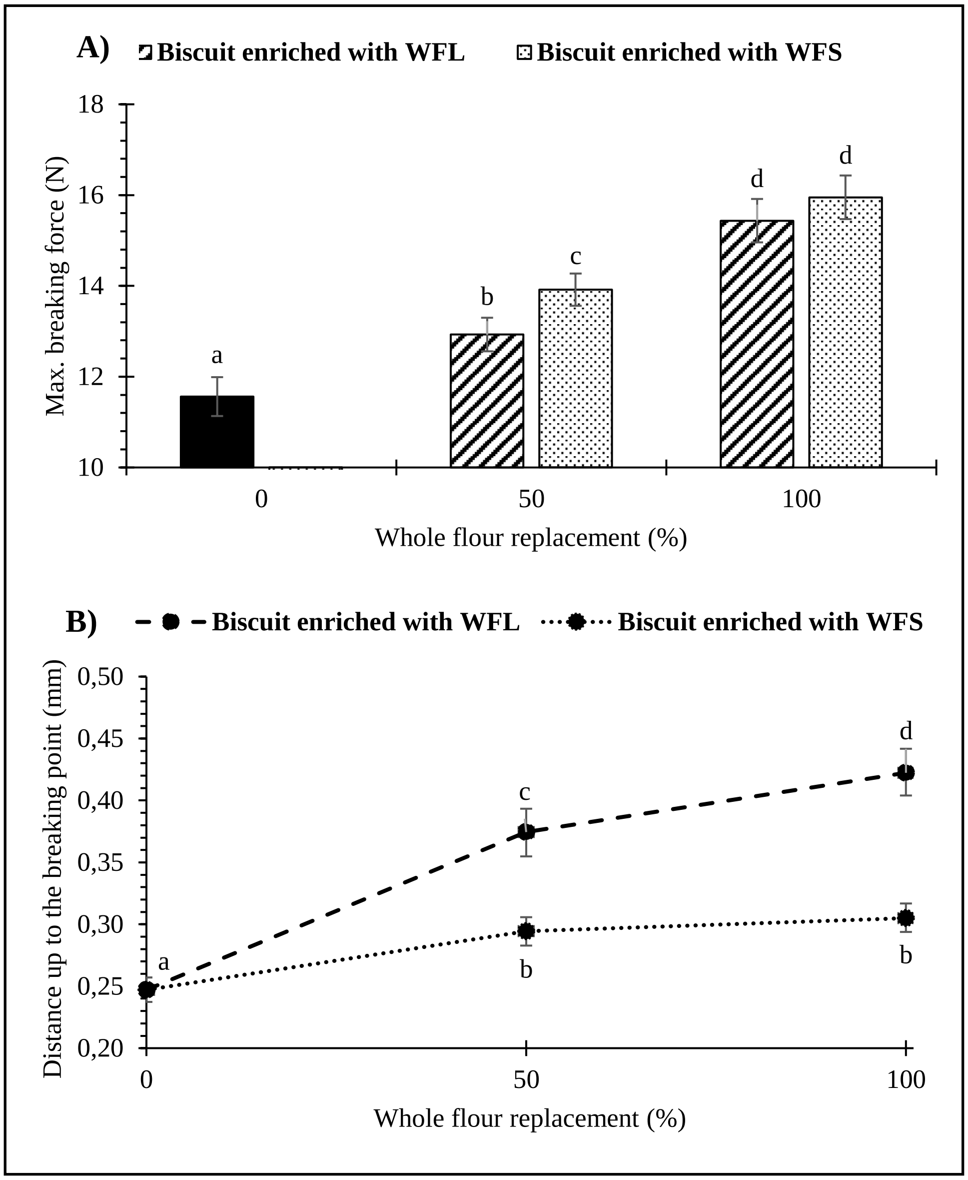


**Figure 4.5** Maximum breaking force [A] and fracturability [B] of biscuits prepared with refined flour and enriched with whole flours containing large or small bran (WFL or WFS) at 50 or 100% of replacement, respectively. Data are observed mean ± confidence intervals at 95%. Different scripts per parameter denote significant differences ($p < 0.05$).

## 4.4 Conclusions

This research focused on the enrichment effect of wheat arabinoxylans and wheat bran with different particle sizes during processing of rotary-moulded biscuits. The results showed that refined flour enriched with wheat bran retained less water than when arabinoxylans were incorporated. In regard to the rheological properties, the firmness of the dough was affected by the wheat bran particle size. Smaller fractions produced a discontinuous and compact structure that increased the overall strength of dough compared to large bran particles. Additionally, the elastic response increased in dough prepared with bran or arabinoxylans, but it was even greater with the latter. This suggests that arabinoxylans contribute to the elasticity of biscuit dough while wheat bran contributes to its stiffness.

Overall, a partial starch gelatinization occurred in rotary-moulded biscuits, and a higher degree of gelatinized starch was obtained in biscuits enriched with arabinoxylans or large bran particles. From a microstructural perspective, it was proposed that the micropores of large wheat bran or water-unextractable arabinoxylan may retain water inside of their capillaries which could be released in a controlled manner during cooking, thus promoting this phenomenon.

## 4.5 Acknowledgements

The authors are grateful to Carla Vallejos for her technical assistance with X-ray computed microtomography, and to Mark Ambühl and Josep Busom for their technical assistance with scanning electron microscopy. Furthermore, the authors would like to acknowledge the financial support of CONICYT through the Advanced National Human Capital Formation Program - Doctoral Fellowship 2016 (Folio No. 21161243), the provision of materials and the technical facilities of Nestlé Development Centre, and FONDEQUIP Project EQM130028.

# 5. FINAL CONCLUSIONS

This thesis aimed to understand the effect of formulation and processing conditions on the quality of rotary-moulded biscuits, by focusing on the impact of creaming stability, sucrose reduction and wheat bran enrichment on the structure development during processing and associated attributes, including the aeration level, the sweetness perception, and the hardness, in order to define some principles to provide healthier options.

Until now, it was generally accepted that the creaming phase of short doughs should be homogeneous and emulsified. Through this thesis, it was possible to conclude that an unstable or stable creaming phase does not modify the quality attributes of rotary-moulded biscuits (with 20% of added sucrose, d.b.) such as hardness, aeration, sweetness, color and noise intensity. The micro-CT analysis revealed that biscuits have a similar microstructure (air porosity and thickness of biscuit walls) when they were prepared with either an unstable or a stable creaming phase.

With respect to sucrose reduction, micro-CT image analysis showed that biscuits with lower levels of sucrose resulted in a microstructure with smaller air pores and thinner biscuit walls, which may be related to a lower degree of grittiness and hardness at the first bite. Furthermore, the results revealed a partial gelatinization of starch granules after baking, and a gelatinization degree that significantly increased from 6% to 40% as the sucrose content decreased from 40% to 10% (d.b.). This behavior may be due to the antiplasticizing effect of the sucrose-water cosolvent, which retarded the sequential thermal events of gelatinization. This effect may also influence the thermal transitions of gluten proteins, and consequently affect the mobility of dough during baking. Controlling the dough expansion has been mainly attributed to starch gelatinization and the denaturation of gluten proteins, which helped to understand the restricted vertical and horizontal expansion during baking in sugar-reduced dough, and the shorter dimensions and the lesser aerated structure in the resultant biscuit.

Regarding rotary-moulded biscuits elaborated with different sucrose particle sizes (granulated or powdered), it was observed that the crystal size did not modify the sweetness perception during chewing when the sucrose content was reduced. Interestingly, biscuits that were formulated with powdered sucrose at a sugar concentration higher than 30% (d.b.), attained an expanded, aerated and non-collapsible structure, which could be linked to the low degree of starch gelatinization. This structure is characterized by having a unique air pore instead of many small ones and, as it has not been seen before in this biscuit category, a patent was filed from this knowledge due to the feasible opportunity to produce a differentiated biscuit texture.

Concerning fiber-enriched biscuits, the isolated incorporation of arabinoxylans, which is the most important source of fiber in wheat bran, was analysed during biscuit-making, as well as the impact of wheat bran inclusion as a whole. Wheat bran had the greatest impact on dough firmness and arabinoxylans had the greatest impact on the elastic response of dough. Also, wheat bran particle size significantly influenced the thermal transition of starch granules during baking. While ~24% of the native starch gelatinized in biscuits with refined or whole flour and small bran, this value increased up to 36% in biscuits enriched with whole flour and large bran. Using a microstructural perspective, the difference in starch gelatinization due to bran particle size was explained by the porous structure of the insoluble part of wheat bran. It was proposed that fiber capillaries may play a key role in enclosing water at micro- and nano-scales, and as heating takes place during baking, the water inside the capillaries of coarse bran could be slowly released, allowing starch granules to partially undergo a thermal transition as cooking progresses. Regarding texture properties in fiber-enriched biscuits, although wheat bran increased the maximum breaking force, a more compressible and less fracturable structure was obtained in biscuits enriched with large bran particles.

From this thesis, it was possible to understand fundamental relationships between formulation, rheology and microstructure of rotary-moulded biscuits, to increase knowledge in the microstructural characterization of this biscuit category, and to get better comprehension of the link between sensory attributes and biscuit properties at (micro) and (macro) structural level. Until now it was generally accepted that the creaming phase of short doughs had to be stable and emulsified in order to obtain an adequate biscuit. This study suggested that the stability of the creaming phase does not seem to be a relevant factor to determine the quality attributes of rotary-moulded biscuits, using a representative formulation in this product category. Furthermore, this research showed that the starch gelatinization increased when the sugar content was reduced. Due to sugar was replace by flour, and considering that this last component has high water-holding capacity, soluble fibers may be an interesting alternative to be used as bulking agents in the biscuit formulation due to their lower water holding capacity compared to flour. Finally, the processing of rotary-moulded dough was not affected by whole flour incorporation. However, the gelatinization of starch granules increased when a porous structure of bran was employed, so that micronized wheat bran could be an alternative to control this phenomenon and, consequently, to reduce the glycemic impact of consuming fiber-enriched biscuits, as could be addressed in future studies. This research may provide a basis for the development of reformulation strategies in rotary-moulded biscuits, which until now have not been studied as much as other biscuit categories, with the purpose to produce healthier biscuits with similar quality attributes as indulgent biscuits offer.

# 6. FUTURE PERSPECTIVES

Further research may focus on the following directions to have a better understanding on how reformulation and processing conditions may modify the quality attributes of rotary-moulded biscuits:

## 6.1 Aeration of Creaming in Rotary-Moulded Biscuits

It has been reported in the literature that the air that is incorporated during the creaming phase may act as bubble nuclei for the leavening agents, thus improving the development of an aerated structure during the baking process (Manley 2011; Brijwani et al., 2008). However, during the moulding stage of rotary-moulded biscuits, the dough is continuously compressed against the forcing roll and into the moulding roll. If an aerated creaming phase undergoes the moulding stage, it could be hypothesized that the air fraction that is entrapped within the creaming phase may be lost due to thecompressive forces that act upon it, affecting the development of an aerated structure during baking. Accordinlgy, a deeper understanting of this phenomenon would be of interest.

## 6.2 Role of Gluten Proteins During Processing of Rotary-Moulded Biscuits

Even though gluten development is highly restricted during rotary-moulded biscuit manufacture, some studies have shown that the thermal transitions of different protein fractions during baking affect the final quality attributes of biscuits (Pareyt et al., 2010a; Pareyt et al., 2010b). It is still necessary to better understand how the *gliadin:glutenin* ratio in soft flour influences the rheological behavior of dough and the quality attributes of biscuits. As an example, Barrera et al. (2007) obtained sugar-snap biscuits with higher diameter:thickness ratio when biscuits were elaborated with strong flour (14% total proteins), but they did not provide any explanation about this counterintuitive result. The study of the relationship between proteins' functionality and gluten development is still incipient, and it could contribute to adequately understand its influence on the final structure of rotary-moulded biscuits.

### 6.3 Sugar Reduction by Incorporating Soluble Fiber as Bulking Agent

From this thesis, it was observed that the reduction of added sucrose by 50%, from 20 to 10% (d.b.), affected negatively dough processability during the moulding stage. The dough tended to dry so quickly due to the high water-holding capacity of flour, so that constant work with external instruments was required to avoid the dough falling from the feeding roll. In addition to this, the degree of gelatinization increased by 12%, which significantly constrained the dough expansion during baking affecting the biscuit's porosity. Soluble fibers may be an interesting alternative to be used as bulking agents instead of adding flour to the biscuit formulation when sugar is reduced. Besides their beneficial physiological effect, including the improvement of intestinal function, cholesterol reduction and the decrease in the risk of type two diabetes (Lattimer & Haub, 2010), studies have shown promising results in biscuit making (Canalis et al., 2019; Mancebo et al., 2018; Mieszkowska & Marzec, 2016). Accordingly, it could be possible to control the dough consistency due to their lower water holding capacity compared to flour.

# 7. REFERENCES


AACC International. (1999). Approved Methods of Analysis, 11th Ed. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A.

Abboud, A. M., & Hoseney, R. C. (1984). Differential scanning calorimetry of sugar cookies and cookie doughs. *Cereal Chemistry*, *61*(1), 34-37.

Aguilera, J.M. (2012). Food Materials and Technology. In J.M. Aguilera (Ed.), *Edible Structures: The Basic Science of What We Eat* (1st ed.). CRC Press: Taylor & Francis Group.

Aguilera, J. M., & Bouchon, P. (2008). Scanning Electron and Transmission Electron Microscopies in Food Analysis. In S. Ötleş (Ed.), *Handbook of food analysis instruments* (1st ed.). CRC Press: Taylor & Francis Group.

Ahmed, J., Almusallam, A. S., Al-Salman, F., AbdulRahman, M. H., & Al-Salem, E. (2013). Rheological properties of water insoluble date fibre incorporated wheat flour dough. *LWT-Food Science and Technology*, *51*(2), 409-416.

Akhtar, S., Khalid, N., Ahmed, I., Shahzad, A., & Suleria, H. A. R. (2014). Physicochemical characteristics, functional properties, and nutritional benefits of peanut oil: a review. *Critical Reviews in Food Science and Nutrition*, *54*(12), 1562-1575.

Allen, T. (2003). Data presentation and interpretation. In T. Allen (Ed.), *Powder sampling and particle size determination* (1st ed.). Elsevier Science.

AOAC International (2012). Official Methods of Analysis of AOAC International, 19th Ed. AOAC International: Gaithersburg, MD, U.S.A.

Baltsavias, A., Jurgens, A., & Vliet, T. V. (1997). Factors affecting fracture properties of short-dough biscuits. *Journal of Texture Studies*, *28*(2), 205-219.

Barak, S., Mudgil, D., & Khatkar, B. S. (2012). Effect of flour particle size and damaged starch on the quality of cookies. *Journal of Food Science and Technology*, *51*(7), 1342-1348.

Barrera, G. N., Pérez, G. T., Ribotta, P. D., & León, A. E. (2007). Influence of damaged starch on cookie and bread-making quality. *European Food Research and Technology*, *225*(1), 1-7.

Biliaderis, C. (2009). Structural transitions and related physical properties of starch. En J. BeMiller, & R. Whistler (Eds.), *Starch: Chemistry and Technology* (2nd ed.). Academic Press.

Biliaderis, C. G. (1983). Differential scanning calorimetry in food research—a review. *Food Chemistry*, *10*(4), 239-265.

Boita, E. R., Oro, T., Bressiani, J., Santetti, G. S., Bertolin, T. E., & Gutkoski, L. C. (2016). Rheological properties of wheat flour dough and pan bread with wheat bran. *Journal of Cereal Science*, *71*, 177-182.

Bordenave, N., Lamothe, L. M., & Kale, M. S. (2020). Dietary Fibers in Foods–Formulating and Processing for Nutritional Benefits. In J. Welti-Chanes, S.O. Serna Saldívar, O.H. Campanella, & V. Tejada-Ortigoza (Eds.), *Science and Technology of Fibers in Food Systems* (pp. 437-457). Springer Science & Business Media

Brijwani, K., Campbell, G. M., & Cicerelli, L. (2008). Aeration of biscuit doughs during mixing. In G.M. Campbell, M.G. Scanlon, & D. Leo Pyle (Eds.), *Bubbles in Food 2* (1st ed.). AACC International, Inc.

Brooker, B. E. (1993). The stabilisation of air in cake batters-the role of fat. *Food Structure, 12*(3), 2.

Bubník, Z., & Kadlec, P. (1995). Sucrose solublity. In M. Mathlouthi, & P. Reiser (Eds.), *Sucrose: Properties and Applications* (1st ed.). Springer Science & Business Media.

Bruker (2019). Bruker micro-CT academy, application method note 059: Porosity analysis [PDF]. https://www.brukersupport.com/Accessed 12 February 2018.

Canalis, M. B., León, A. E., & Ribotta, P. D. (2019). Incorporation of dietary fiber on the cookie dough. Effects on thermal properties and water availability. *Food Chemistry, 271*, 309-317.

Canalis, M. B., Valentinuzzi, M. C., Acosta, R. H., León, A. E., & Ribotta, P. D. (2018). Effects of Fat and Sugar on Dough and Biscuit Behaviours and their Relationship to Proton Mobility Characterized by TD-NMR. *Food and Bioprocess Technology, 11*(5), 953-965.

Capek, I. (2004). Degradation of kinetically-stable o/w emulsions. *Advances in Colloid and Interface Science*, *107*(2-3), 125-155.

Chevallier, S., Della Valle, G., Colonna, P., Broyart, B., & Trystram, G. (2002). Structural and chemical modifications of short dough during baking. *Journal of Cereal Science*, *35*(1), 1-10.

Chevallier, S., Colonna, P., Buléon, A., & Della Valle, G. (2000a). Physicochemical behaviors of sugars, lipids, and gluten in short dough and biscuit. *Journal of Agricultural and Food Chemistry*, *48*(4), 1322-1326.

Chevallier, S., Colonna, P., Della Valle, G., & Lourdin, D. (2000b). Contribution of major ingredients during baking of biscuit dough systems. *Journal of Cereal Science*, *31*(3), 241-252.

Chiotelli, E., Rolée, A., & Le Meste, M. (2000). Effect of sucrose on the thermomechanical behavior of concentrated wheat and waxy corn starch− water preparations. *Journal of Agricultural and Food Chemistry*, *48*(4), 1327-1339.

Colakoglu, A. S., & Özkaya, H. (2012). Potential use of exogenous lipases for DATEM replacement to modify the rheological and thermal properties of wheat flour dough. *Journal of Cereal Science*, *55*(3), 397-404.

Colombo, A., Pérez, G. T., Ribotta, P. D., & León, A. E. (2008). A comparative study of physicochemical tests for quality prediction of Argentine wheat flours used as corrector flours and for cookie production. *Journal of Cereal Science*, *48*(3), 775-780.

Colonna, P. & Buleon, A. (2010). Thermal transitions of starches. In A.C. Bertolini (Ed.), *Starches: Characterization, Properties and Applications* (1st ed.). CRC Press.

Contamine, A. S., Abecassis, J., Morel, M.-H., Vergnes, B., & Verel, A. (1995). Effect of mixing conditions on the quality of dough and biscuits. *Cereal Chemistry*, *72*(6), 516-522.

Contardo, I., & Bouchon, P. (2018). Enhancing Micro-CT methods to quantify oil content and porosity in starch-gluten matrices. *Journal of Food Engineering*, *237*, 154-161.

Contardo, I., Parada, J., Leiva, A., & Bouchon, P. (2016). The effect of vacuum frying on starch gelatinization and its in vitro digestibility in starch–gluten matrices. *Food Chemistry*, *197*, 353-358.

Courtin, C. M., & Delcour, J. A. (2002). Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*, *35*(3), 225-243.

Curley, L. P. & Hoseney, R. C. (1984). Effects of Corn Sweeteners on Cookie Quality. *Cereal Chemistry, 61*, 274-278.

Damodaran, S. (1996). Amino Acids, Peptides, and Proteins. In R.O. Fennema (Ed.), *Food Chemistry* (3rd ed.). CRC Press.

Davis, E. A. (1995). Functionality of sugars: physicochemical interactions in foods. *The American Journal of Clinical Nutrition*, *62*(1), 170S-177S.

Davidson, I. (2018). Dough Mixing. In I. Davidson (Ed.), *Biscuit, cookie and cracker production: process, production and packaging equipment* (1st ed.). Academic Press.

Devi, A., & Khatkar, B. S. (2018). Effects of fatty acids composition and microstructure properties of fats and oils on textural properties of dough and cookie quality. *Journal of Food Science and Technology*, *55*(1), 321-330.

Devi, A., & Khatkar, B. S. (2016). Physicochemical, rheological and functional properties of fats and oils in relation to cookie quality: a review. *Journal of Food Science and Technology*, *53*(10), 3633-3641.

Doescher, L. C., Hoseney, R. C., & Milliken, G. A. (1987a). A mechanism for cookie dough setting. *Cereal Chemistry*, *64*(3), 158-163.

Doescher, L. C., Hoseney, R. C., Milliken, G. A., & Rubenthaler, G. L. (1987b). Effect of sugars and flours on cookie spread evaluated by time-lapse photography. *Cereal Chemistry, 64*(3), 163-7.

Doescher, L. C., & Hoseney, R. C. (1985). Effect of sugar type and flour moisture on surface cracking of sugar-snap cookies. *Cereal Chemistry, 62*, 263-266.

Dornez, E., Holopainen, U., Cuyvers, S., Poutanen, K., Delcour, J. A., Courtin, C. M., & Nordlund, E. (2011). Study of grain cell wall structures by microscopic analysis with four different staining techniques. *Journal of Cereal Science*, *54*(3), 363-373.

Duyvejonck, A. E., Lagrain, B., Pareyt, B., Courtin, C. M., & Delcour, J. A. (2011). Relative contribution of wheat flour constituents to solvent retention capacity profiles of European wheats. *Journal of Cereal Science*, *53*(3), 312-318.

Efron, B. (1992). Bootstrap methods: another look at the jackknife. In S. Kotz, & N.L. Johnson, *Breakthroughs in statistics* (1st ed.). Springer Science & Business Media.

European Food Information Council (EUFIC) (2018). Global update on nutrition labelling (Executive Summary). https://www.eufic.org/images/uploads/healthy-living/Executive-Summary-GUNL-2018-V2.pdf/Accessed 12 June 2020.

Feldkamp, L. A., Davis, L. C., & Kress, J. W. (1984). Practical cone-beam algorithm. *Josa a*, *1*(6), 612-619.

Filipčev, B., Nedeljković, N., Šimurina, O., Sakač, M., Pestorić, M., Jambrec, D., ... & Jovanov, P. (2017). Partial replacement of fat with wheat bran in formulation of biscuits enriched with herbal blend. *Hemijska Industrija*, *71*(1), 61-67.

Frisullo, P., Conte, A., & Del Nobile, M. A. (2010). A Novel Approach to Study Biscuits and Breadsticks Using X-Ray Computed Tomography. *Journal of Food Science*, *75*(6), E353-E358.

Games, P. A., Keselman, H. J., & Clinch, J. J. (1979). Tests for homogeneity of variance in factorial designs. *Psychological Bulletin*, *86*(5), 978.

Ghiasi, K., & RC, H. (1983). Effects of flour components and dough ingredients on starch gelatinization. *Cereal Chemistry*, *60*(1), 58-61.

Ghotra, B. S., Dyal, S. D., & Narine, S. S. (2002). Lipid shortenings: a review. *Food Research International*, *35*(10), 1015-1048.

Glibowski, P., Zarzycki, P., & Krzepkowska, M. (2008). The rheological and instrumental textural properties of selected table fats. *International Journal of Food Properties*, *11*(3), 678-686.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology*, *16*(1-3), 12-30.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J., & Joy, D. C. (2018). In J.I. Goldstein, D.E. Newbury, J.R. Michael, N.W. Ritchie, J.H.J. Scott, & D.C. Joy (Eds.), *Scanning electron microscopy and X-ray microanalysis* (4th ed.). Springer: Science & Business Media.

Gupta, R. B., Khan, K., & Macritchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science*, *18*(1), 23-41.

Hartel, R. W., Ergun, R., & Vogel, S. (2011). Phase/state transitions of confectionery sweeteners: Thermodynamic and kinetic aspects. *Comprehensive Reviews in Food Science and Food Safety*, *10*(1), 17-32.

Hartel, R. W. (2001). Nucleation. In R.W. Hartel (Ed.), *Crystallization in foods* (1st ed.). Aspen Publishers, Inc.

Hemdane, S., Langenaeken, N. A., Jacobs, P. J., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2018). Study of the role of bran water binding and the steric hindrance by bran in straight dough bread making. *Food Chemistry*, *253*, 262-268.

Holopainen-Mantila, U., & Raulio, M. (2016). Cereal Grain Structure by Microscopic Analysis. In N. Sozer (Ed.), *Imaging Technologies and Data Processing for Food Engineers* (1st ed.). Springer Science & Business Media.

Jacobs, P. J., Hemdane, S., Dornez, E., Delcour, J. A., & Courtin, C. M. (2015). Study of hydration properties of wheat bran as a function of particle size. *Food Chemistry*, *179*, 296-304.

Jacob, J., & Leelavathi, K. (2007). Effect of fat-type on cookie dough and cookie quality. *Journal of Food Engineering*, *79*(1), 299-305.

Jambrec, D., Pestorić, M., Sakač, M., Nedeljković, N., Hadnađev, M., Filipčev, B., & Šimurina, O. (2013). Sensory and instrumental properties of novel gluten-free products. *Journal on Processing and Energy in Agriculture*, *17*(2), 86-88.

Jribi, S., Sahagún, M., Belorio, M., Debbabi, H., & Gomez, M. (2020). Effect of sprouting time on dough and cookies properties. *Journal of Food Measurement and Characterization*, 1-6.

Kamel, B. S. (1994). Creaming, emulsions, and emulsifiers. In H. Faridi (Ed.), *The science of cookie and cracker production* (1st ed.). Chapman & Hall.

Kim, E. H. J., Corrigan, V. K., Wilson, A. J., Waters, I. R., Hedderley, D. I., & Morgenstern, M. P. (2012). Fundamental fracture properties associated with sensory hardness of brittle solid foods. *Journal of Texture Studies*, *43*(1), 49-62.

Kiszonas, A. M., Courtin, C. M., & Morris, C. F. (2012). A critical assessment of the quantification of wheat grain arabinoxylans using a phloroglucinol colorimetric assay. *Cereal Chemistry*, *89*(3), 143-150.

Kohyama, K., Hayakawa, F., Kazami, Y., & Nishinari, K. (2016). Sucrose release from agar gels and sensory perceived sweetness. *Food Hydrocolloids*, *60*, 405-414.

Kuktaite, R., Larsson, H., Marttila, S., & Johansson, E. (2005). Effect of mixing time on gluten recovered by ultracentrifugation studied by microscopy and rheological measurements. *Cereal Chemistry*, *82*(4), 375-384.

Kweon, M., Slade, L., Levine, H., & Gannon, D. (2014). Cookie-versus cracker-baking—what's the difference? Flour functionality requirements explored by src and alveography. *Critical Reviews in Food Science and Nutrition*, *54*(1), 115-138.

Kweon, M., Slade, L., & Levine, H. (2011). Solvent retention capacity (SRC) testing of wheat flour: Principles and value in predicting flour functionality in different wheat-based food processes and in wheat breeding—A review. *Cereal Chemistry*, *88*(6), 537-552.

Kweon, M., Slade, L., Levine, H., Martin, R., & Souza, E. (2009). Exploration of sugar functionality in sugar-snap and wire-cut cookie baking: Implications for potential sucrose replacement or reduction. *Cereal Chemistry*, *86*(4), 425-433.

Laguna, L., Vallons, K. J., Jurgens, A., & Sanz, T. (2013a). Understanding the effect of sugar and sugar replacement in short dough biscuits. *Food and Bioprocess Technology*, *6*(11), 3143-3154.

Laguna, L., Primo-Martín, C., Salvador, A., & Sanz, T. (2013b). Inulin and erythritol as sucrose replacers in short-dough cookies: sensory, fracture, and acoustic properties. *Journal of Food Science, 78*(5), S777-S784.

Laguna, L., Salvador, A., Sanz, T., & Fiszman, S. M. (2011). Performance of a resistant starch rich ingredient in the baking and eating quality of short-dough biscuits. *LWT-Food Science and Technology, 44*(3), 737-746.

Lapčíková, B., Burešová, I., Lapčík, L., Dabash, V., & Valenta, T. (2019). Impact of particle size on wheat dough and bread characteristics. *Food Chemistry, 297*, 124938.

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients*, *2*(12), 1266-1289.

Lawless, H. T., & Heymann, H. (2010). Time-Intensity Methods. In H.T. Lawless, & H. Heymann (Eds.), *Sensory evaluation of food: principles and practices*. Springer Science & Business Media.

Le Bleis, F., Chaunier, L., Chiron, H., Della Valle, G., & Saulnier, L. (2015). Rheological properties of wheat flour dough and French bread enriched with wheat bran. *Journal of Cereal Science*, *65*, 167-174.

Le Calvé, B., Saint-Léger, C., Gaudreau, N., & Cayeux, I. (2019). Capturing key sensory moments during biscuit consumption: Using TDS to evaluate several concurrent sensory modalities. *Journal of Sensory Studies*, *34*(6), e12529.

León, A. E., Barrera, G. N., Pérez, G. T., Ribotta, P. D., & Rosell, C. M. (2006). Effect of damaged starch levels on flour-thermal behaviour and bread staling. *European Food Research and Technology*, *224*(2), 187-192.

Létang, C., Piau, M., & Verdier, C. (1999). Characterization of wheat flour–water doughs. Part I: Rheometry and microstructure. *Journal of Food Engineering*, *41*(2), 121-132.

Li, X., Hu, H., Xu, F., Liu, Z., Zhang, L., & Zhang, H. (2019). Effects of aleurone-rich fraction on the hydration and rheological properties attributes of wheat dough. *International Journal of Food Science & Technology*, *54*(5), 1777-1786.

Li, J., Hou, G. G., Chen, Z., Chung, A. L., & Gehring, K. (2014). Studying the effects of whole-wheat flour on the rheological properties and the quality attributes of whole-wheat saltine cracker using SRC, alveograph, rheometer, and NMR technique. *LWT-Food Science and Technology*, *55*(1), 43-50.

Lv, M. (2018). *An analytical and experimental study on the onset of sand production: A poro-elastoplastic model with strain softening behavior* (Doctoral dissertation). The University of New South Wales, Sydney, Australia.

Maache-Rezzoug, Z., Bouvier, J. M., Allaf, K., & Patras, C. (1998). Effect of principal ingredients on rheological behaviour of biscuit dough and on quality of biscuits. *Journal of Food Engineering*, *35*(1), 23-42.

Maes, C., & Delcour, J. A. (2002). Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *Journal of Cereal Science*, *35*(3), 315-326.

Mamat, H., & Hill, S. E. (2014). Effect of fat types on the structural and textural properties of dough and semi-sweet biscuit. *Journal of Food Science and Technology*, *51*(9), 1998-2005.

Mancebo, C. M., Rodríguez, P., Martínez, M. M., & Gómez, M. (2018). Effect of the addition of soluble (nutriose, inulin and polydextrose) and insoluble (bamboo, potato and pea) fibres on the quality of sugar-snap cookies. *International Journal of Food Science & Technology*, *53*(1), 129-136.

Manley, D. (2011). In D. Manley (Ed.), *Manley's technology of biscuits, crackers and cookies* (4th ed.). Woodhead Publishing Limited.

Manohar, R. S., & Rao, P. H. (1999). Effect of mixing method on the rheological characteristics of biscuit dough and the quality of biscuits. *European Food Research and Technology*, *210*(1), 43-48.

Manohar, R. S., & Rao, P. H. (1997). Effect of sugars on the rheological characteristics of biscuit dough and quality of biscuits. *Journal of the Science of Food and Agriculture*, *75*(3), 383-390.

Maskan, M., & Göğüş, F. (2000). Effect of sugar on the rheological properties of sunflower oil–water emulsions. *Journal of Food Engineering*, *43*(3), 173-177.

Meyners, M., Carr, B. T., & Hasted, A. (2020). To replicate or not to replicate, or when did we start to ignore the concept of statistical power? *Food Quality and Preference*, *79*, 103633.

Mieszkowska, A., & Marzec, A. (2016). Effect of polydextrose and inulin on texture and consumer preference of short-dough biscuits with chickpea flour. *LWT-Food Science and Technology*, *73*, 60-66.

Molina, M.T., M. Vaz, S., Leiva, A., Bouchon, P. (2020). Rotary-moulded biscuits: Dough expansion, microstructure and sweetness perception as affected by sucrose content and its particle size. *Food Research International* (Submitted).

Molina, M. T., Leiva, A., & Bouchon, P. (2016). Examining the effect of freezing on starch gelatinization during heating at high rates using online in situ hot-stage video-microscopy and differential scanning calorimetry. *Food and Bioproducts Processing*, *100*, 488-495.

Monteleone, E., Condelli, N., Dinnella, C., & Bertuccioli, M. (2004). Prediction of perceived astringency induced by phenolic compounds. *Food Quality and Preference*, *15*(7-8), 761-769.

Moser, M., Lepage, M., Pineau, N., Fillion, L., & Rytz, A. (2018). Replicates in sensory profiling: Quantification of the impact of moving from two to one assessments. *Food Quality and Preference*, *65*, 185-190.

Nandeesh, K., Jyotsna, R., & Venkateswara Rao, G. (2011). Effect of differently treated wheat bran on rheology, microstructure and quality characteristics of soft dough biscuits. *Journal of Food Processing and Preservation*, *35*(2), 179-200.

Navidi, W. C. (2011). Confidence Intervals. In W.C. Navidi (Ed.), *Statistics for engineers and scientists* (3rd ed.). McGraw-Hill Higher Education.

O'Brien, R. D. (2008). In R.D. O'Brien (Ed.), *Fats and oils: formulating and processing for applications* (1st ed.). CRC press: Taylor & Francis Group.

Onipe, O. O., Jideani, A. I., & Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. *International Journal of Food Science & Technology*, *50*(12), 2509-2518.

Pareyt, B., Goovaerts, M., Broekaert, W. F., & Delcour, J. A. (2011). Arabinoxylan oligosaccharides (AXOS) as a potential sucrose replacer in sugar-snap cookies. *LWT-Food Science and Technology*, *44*(3), 725-728.

Pareyt, B., Van Steertegem, B., Brijs, K., Lagrain, B., & Delcour, J. A. (2010a). The impact of redox agents on sugar-snap cookie making. *Journal of Cereal Science*, *52*(2), 192-199.

Pareyt, B., Brijs, K., & Delcour, J. A. (2010b). Impact of Fat on Dough and Cookie Properties of Sugar-Snap Cookies. *Cereal Chemistry*, *87*(3), 226-230.

Pareyt, B., Talhaoui, F., Kerckhofs, G., Brijs, K., Goesaert, H., Wevers, M., & Delcour, J. A. (2009a). The role of sugar and fat in sugar-snap cookies: Structural and textural properties. *Journal of Food Engineering*, *90*(3), 400-408.

Pareyt, B., Brijs, K., & Delcour, J. A. (2009b). Sugar-snap cookie dough setting: the impact of sucrose on gluten functionality. *Journal of Agricultural and Food Chemistry*, *57*(17), 7814-7818.

Pareyt, B., & Delcour, J. A. (2008a). The role of wheat flour constituents, sugar, and fat in low moisture cereal based products: a review on sugar-snap cookies. *Critical Reviews in Food Science and Nutrition*, *48*(9), 824-839.

Pareyt, B., Wilderjans, E., Goesaert, H., Brijs, K., & Delcour, J. A. (2008b). The role of gluten in a sugar-snap cookie system: A model approach based on gluten–starch blends. *Journal of Cereal Science*, *48*(3), 863-869.

Pasha, I., Anjum, F. M., & Butt, M. S. (2009). Genotypic variation of spring wheats for solvent retention capacities in relation to end-use quality. *LWT-Food Science and Technology*, *42*(1), 418-423.

Perry, P. A., & Donald, A. M. (2002). The effect of sugars on the gelatinisation of starch. *Carbohydrate Polymers*, *49*(2), 155-165.

Ram, S., & Singh, R. P. (2004). Solvent retention capacities of Indian wheats and their relationship with cookie-making quality. *Cereal Chemistry*, *81*(1), 128-133.

Ramseyer, D. D., Bettge, A. D., & Morris, C. F. (2011). Distribution of Total, Water-Unextractable, and Water-Extractable Arabinoxylans in Wheat Flour Mill Streams. *Cereal Chemistry*, *88*(2), 209-216.

Rizzo, M. L. (2007). Bootstrap and Jackknife. In M.L. Rizzo (Ed.), *Statistical computing with R* (1st ed.). Chapman & Hall/CRC Press.

Rodríguez-García, J., Laguna, L., Puig, A., Salvador, A., & Hernando, I. (2013). Effect of fat replacement by inulin on textural and structural properties of short dough biscuits. *Food and Bioprocess Technology*, *6*(10), 2739-2750.

Roozendaal, H., Abu-hardan, M., & Frazier, R. A. (2012). Thermogravimetric analysis of water release from wheat flour and wheat bran suspensions. *Journal of Food Engineering*, *111*(4), 606-611.

Sabanis, D., Lebesi, D., & Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *LWT-Food Science and Technology*, *42*(8), 1380-1389.

Santana, R. C., Perrechil, F. A., & Cunha, R. L. (2013). High-and low-energy emulsifications for food applications: a focus on process parameters. *Food Engineering Reviews*, *5*(2), 107-122.

Saulnier, L., Sado, P. E., Branlard, G., Charmet, G., & Guillon, F. (2007). Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties. *Journal of Cereal Science*, *46*(3), 261-281.

Schoeman, L., Williams, P., du Plessis, A., & Manley, M. (2016). X-ray micro-computed tomography (μCT) for non-destructive characterisation of food microstructure. *Trends in Food Science & Technology*, *47*, 10-24.

Shannon, J. & Garwood, D.L. (2009). Genetics and Physiology of Starch Development. In J. BeMiller, & R. Whistler (Eds.), *Starch: Chemistry and Technology* (3rd ed.). Food Science and Technology, International Series.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. *Current Atherosclerosis Reports*, *12*(6), 384-390.

Slade, L., & Levine, H. (1994). Structure-function relationships of cookie and cracker ingredients. In H. Faridi (Ed.), *The science of cookie and cracker production* (1st ed.). Chapman & Hall.

Slade, L., & Levine, H. (1987). Recent advances in starch retrogradation. In S.S. Stivala, V. Crescenzi, & I.C.M. Dea (Eds.), *Industrial polysaccharides – The impact of Biotechnology and Advanced Methodologies* (1st ed.). CRC Press.

Sozer, N., Cicerelli, L., Heiniö, R. L., & Poutanen, K. (2014). Effect of wheat bran addition on in vitro starch digestibility, physico-mechanical and sensory properties of biscuits. *Journal of Cereal Science*, *60*(1), 105-113.

Struck, S., Jaros, D., Brennan, C. S., & Rohm, H. (2014). Sugar replacement in sweetened bakery goods. *International Journal of Food Science & Technology*, *49*(9), 1963-1976.

Sudha, M. L., Vetrimani, R., & Leelavathi, K. (2007). Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chemistry*, *100*(4), 1365-1370.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, *108*, 303-318.

Tarancón, P., Salvador, A., Sanz, T., Fiszman, S., & Tárrega, A. (2015). Use of healthier fats in biscuits (olive and sunflower oil): changing sensory features and their relation with consumers' liking. *Food Research International*, *69*, 91-96.

Tarancón, P., Sanz, T., Salvador, A., & Tárrega, A. (2014). Effect of fat on mechanical and acoustical properties of biscuits related to texture properties perceived by consumers. *Food and Bioprocess Technology*, *7*(6), 1725-1735.

Tiefenbacher, K. F. (2017). In K.F. Tiefenbacher (Ed.), *Wafer and Waffle: Processing and Manufacturing* (1st ed.). Elsevier Inc.

Yılmaz, E., & Öğütcü, M. (2015). The texture, sensory properties and stability of cookies prepared with wax oleogels. *Food & Function, 6*(4), 1194-1204.

Van Craeyveld, V. (2009). *Production and functional characterisation of arabinoxylan-oligosaccharides from wheat (Triticum aestivum L.) bran and psyllium (Plantago ovata Forsk) seed husk* (Doctoral dissertation). Katholieke Universiteit Leuven, Leuven, Belgium.

van der Sman, R. G. M., & Renzetti, S. (2018). Understanding functionality of sucrose in biscuits for reformulation purposes. *Critical Reviews in Food Science and Nutrition*, *59*(14), 2225-2239.

Wang, N., Hou, G. G., Kweon, M., & Lee, B. (2016). Effects of particle size on the properties of whole-grain soft wheat flour and its cracker baking performance. *Journal of Cereal Science, 69*, 187-193.

Yang, N., Fisk, I. D., Linforth, R., Brown, K., Walsh, S., Mooney, S., ... & Hort, J. (2012). Impact of flavour solvent on biscuit micro-structure as measured by X-ray micro-computed tomography and the distribution of vanillin and HMF (HPLC). *European Food Research and Technology*, *235*(6), 1083-1091.