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Table of contents

ACKNOWLEDGMENT **3**

TABLE OF CONTENTS **5**

ABBREVIATIONS AND SYMBOLS OF MEASUREMENT **11**

LIST OF TABLES **14**

LIST OF FIGURES **16**

INTRODUCTION **23**

1. STATE OF THE ART **25**

1.1. BISCUITS 26

1.1.1. Biscuits among other cereal products *26*
1.1.2. Classification of biscuits *27*
1.1.3. Biscuit sector in France *28*
1.1.4. Biscuit constituent *29*

1.1.4.1. Soft wheat flour 29

1.1.4.1.1. Primary components of flour: starch 31
1.1.4.1.2. Primary components of flour: protein 32

1.1.4.2. Sugar 34
1.1.4.3. Fat 35
1.1.4.4. Water 35
1.1.4.5. Leavening agents (LAs) 35

1.1.5. Minor ingredients *37*

1.1.5.1. Glucose syrup 37
1.1.5.2. Salt 37
1.1.5.3. Dairy ingredients 37
1.1.5.4. L-Cysteine 38

1.2. FABRICATION OF BISCUITS 39

1.2.1. Mixing *39*
1.2.2. Forming *40*
1.2.3. Baking /cooling *41*

1.2.3.1. Conventional baking 41
1.2.3.2. Energy consumption and environmental impact of baking 42
1.2.3.3. Alternative baking technologies 44

1.2.3.3.1. Microwave baking of biscuits 46
1.2.3.3.2. Fundamentals of microwave heating 47
1.2.3.3.3. Dielectric properties of foods 49
1.2.3.3.4. Effect of microwave on starch structure 51

1.3. QUALITY OF BISCUITS 52

1.3.1. Physical parameters *52*

1.3.1.1. Appearance 52

1.3.1.2. Water content 52
1.3.1.3. Dimension 52
1.3.1.4. Specific volume 53
1.3.1.5. Texture 53
1.3.1.6. Porosity 53
1.3.1.7. Colour 54
1.3.2. Biochemical parameters 54
1.3.2.1. pH 54
1.3.2.2. Starch content 54
1.3.3. Nutrition parameters 55
1.3.3.1. Dietary fibres and their impact on dough 55
1.3.3.2. Glycaemic index (GI) and impact of baking on it 56
1.3.4. Neo-formed contaminants (NFCs) 60
1.4. INNOVATION IN BISCUIT MANUFACTURING 61
1.4.1. Formulation 61
1.4.2. Mitigation of acrylamide (AA) 63
1.4.2.1. Ammonium bicarbonate (ABC) substitution with sodium bicarbonate (SBC) 63
1.4.2.2. Replacing leavening agents (LAs) with CO_2 mixing 63
1.4.2.3. Process modification 64
1.5. STATE OF THE ART'S CONCLUSION 66
2. THE CONTEXT, POSITIONING, AND SCIENTIFIC APPROACH 67
2.1. PROJECT CONTEXT AND POSITIONING 68
2.2. SCIENTIFIC PROBLEM AND RESEARCH QUESTIONS 69
2.3. PROJECT OBJECTIVES AND SCIENTIFIC APPROACH 70
3. MATERIALS AND METHODS 73
3.1. SELECTION OF FLOUR, OPTIMAL MIXING, AND BAKING CONDITIONS 74
3.1.1. Primary ingredients for biscuit dough preparation 74
3.1.1.1. Flour 74
3.1.1.2. Additional ingredients 74
3.1.2. Formulation 74
3.1.3. Mixing procedure 76
3.1.3.1. Laminated biscuit dough mixing procedure 76
3.1.3.2. Void fraction of gas in dough 80
3.1.3.2.1. Apparent density 80
3.1.3.2.2. Real density 81
3.1.3.2.3. Void fraction of gas in dough 81
3.1.4. Rotary biscuit dough mixing procedure 82
3.1.5. Forming procedure 82
3.1.5.1. Laminated biscuit forming procedure 82
3.1.5.2. Rotary biscuit forming procedure 83
3.1.6. Baking procedure 83
3.1.6.1.1. Optimal Conventional baking of laminated biscuits 83

3.1.6.1.2. Optimal conventional baking of rotary biscuits 84
3.1.7. Kinetics of mass loss during baking 85
3.1.8. Thermophysical properties: freezable water 85
3.2. IMPACT OF REMOVING LEAVENING AGENTS (LAS) FROM LAMINATED BISCUITS 86
3.2.1. Characterisation methods 86
3.2.1.1. Dough characteristics 86
3.2.1.1.1. pH 86
3.2.1.1.2. Void fraction of gas in dough 86
3.2.1.2. Biscuit characteristics 86
3.2.1.2.1. Porosity 86
3.2.1.2.2. Water content 87
3.2.1.2.3. Colour 87
3.2.1.2.4. Texture 88
3.3. MICROWAVE BAKING OF BISCUITS AS AN ALTERNATIVE TO CONVENTIONAL BAKING 89
3.3.1. Microwave baking 89
3.3.1.1. Dielectric properties of non-baked biscuits 89
3.3.1.2. Microwave cavity characterisation 90
3.3.1.3. Microwave baking procedure 92
3.3.1.4. Energy consumption in microwave baking of biscuits 93
3.3.1.4.1. Evaluation of energy efficiency of the microwave oven 93
3.3.1.4.2. Baking energy of biscuits 95
3.4. IMPACT OF BISCUIT FORMULATION AND BAKING ON ACRYLAMIDE (AA) FORMATION 96
3.4.1. Biscuit preparation 96
3.4.2. Characterisation methods 97
3.4.3. Acrylamide (AA) analysis 98
3.4.3.1. Chemicals for analysis 98
3.4.3.2. Acrylamide (AA) extraction 98
3.4.3.3. Extract purification by solid phase extraction (SPE) 99
3.4.3.4. LC-MS/MS analysis of acrylamide (AA) 99
3.4.4. Sensory analysis 100
3.4.4.1. General conditions 100
3.4.4.1.1. Ethical statement 100
3.4.4.1.2. Sensory analysis laboratory 100
3.4.4.1.3. Panellists 100
3.4.4.1.4. Experimental conditions 100
3.4.4.2. Study of the influence of biscuit formulation and baking method on panellists' perception 101
3.4.4.2.1. Hedonic test 101
3.4.4.2.2. Check-All-That-Apply (CATA) test 101
3.5. *IN VITRO* STARCH DIGESTIBILITY AND ESTIMATED GLYCAEMIC INDEX (EGI) 102
3.5.1. Estimated glycaemic index (eGI) 102
3.5.1.1. Resistant (RS) and digestible starch (DS) 102
3.5.1.2. Total starch (TS) 102
3.5.1.3. *In vitro* starch digestion 102

3.5.1.3.1. Preparation of buffers and enzymes 102
3.5.1.3.2. Sample preparation and kinetics of starch digestion 104
3.5.1.3.3. Estimated glycaemic index (eGI) of biscuits 104

3.6. FIBRE-ADDED LAMINATED BISCUITS 106

3.6.1. Primary ingredients for fibre-added biscuit 106
3.6.2. Physical and functional properties of acorn and carob flours 107

3.6.2.1. Water content 107
3.6.2.2. Colour 107
3.6.2.3. Bulk density 107
3.6.2.4. Water holding capacity (WHC) 107
3.6.2.5. Oil holding capacity (OHC) 108
3.6.2.6. Swelling capacity (SWC) 108

3.6.3. Formulation 108
3.6.4. Mixing procedure and the dough texture 109
3.6.5. Forming procedure 110
3.6.6. Baking procedure 110
3.6.7. Characterisation methods 111
3.6.8. Sensory analysis 111

3.6.8.1.1. Hedonic test 111
3.6.8.1.2. Just about right (JAR) test 112

3.7. SIMPLIFIED MASS-ENERGY BALANCE APPROACH FOR BISCUIT BAKING 113
3.8. STATISTICAL ANALYSIS 118

4. RESULTS AND DISCUSSION 119

4.1. SELECTION OF FLOUR, OPTIMAL MIXING, AND BAKING CONDITIONS 120

4.1.1. Selection of flour 120
4.1.2. Optimal mixing conditions for laminated biscuit dough containing leavening agents (LAs) 121
4.1.3. Optimal mixing conditions for CO_2 over-pressure mixing of leavening agents-free (LAs-free) laminated biscuit dough 122
4.1.4. Optimal baking conditions for laminated biscuits 124
4.1.5. Comparison of leavening agents-free (LAs-free) laminated biscuits mixed under CO_2 over-pressure with and without gas flow 127
4.1.6. Freezable water in the laminated biscuit dough 128
4.1.7. Conclusion 128

4.2. ARTICLE 1: IMPACT OF REMOVING LEAVENING AGENTS FROM LAMINATED BISCUITS 130

4.2.1. Introduction 131
4.2.2. Materials and methods 134

4.2.2.1. Materials 134
4.2.2.2. Mixing and dough preparation 134
4.2.2.3. Biscuit dough sheeting and shaping 136
4.2.2.4. Baking 136
4.2.2.5. Analysis of dough and biscuits 137

4.2.2.5.1. Dough pH 137
4.2.2.5.2. Void fraction of gas in dough 137
4.2.2.5.3. Morphology and apparent density of biscuits 137
4.2.2.5.4. Biscuit porosity 137
4.2.2.5.5. Water content of biscuits 138

4.2.2.5.6. Colour of biscuits 138
4.2.2.5.7. Texture of biscuits 138
4.2.2.5.8. Statistical analysis 138

4.2.3. Results and discussion 139

4.2.3.1. Dough properties 139

4.2.3.1.1. Dough pH 139
4.2.3.1.2. Void fraction of gas in dough 139
4.2.3.1.3. Morphology, apparent density, and porosity of biscuits 140
4.2.3.1.4. Water content of biscuits 141
4.2.3.1.5. Colour of biscuits 142
4.2.3.1.6. Texture of biscuits 144

4.2.4. Conclusion 144
4.2.5. Author contributions 145
4.2.6. Acknowledgment 145
4.2.7. References 145

4.3. MICROWAVE BAKING OF BISCUITS AS AN ALTERNATIVE TO CONVENTIONAL BAKING 148

4.3.1. Dielectric properties of the dough 148
4.3.2. Optimal sample position for microwave baking of the laminated and rotary biscuits 149
4.3.3. Microwave baking of laminated biscuits 152
4.3.4. Microwave baking of rotary biscuits 154
4.3.5. Microwave energy consumption 156

4.3.5.1. Efficiency of the microwave oven 156
4.3.5.2. Baking energy 158

4.3.6. Conclusion 158

4.4. ARTICLE 2: IMPACT OF BISCUIT FORMULATION AND BAKING ON ACRYLAMIDE FORMATION 160

4.4.1. Introduction 161
4.4.2. Materials and Methods 164

4.4.2.1. Materials 164

4.4.2.1.1. Biscuit ingredients 164
4.4.2.1.2. Chemicals for Acrylamide Analysis 165

4.4.2.2. Biscuit Preparation 165

4.4.2.2.1. Biscuits Formulation 165
4.4.2.2.2. Dough Mixing, Shaping, and Baking 166

4.4.2.3. Dough and Biscuit Quality 167

4.4.2.3.1. Dough pH 167
4.4.2.3.2. Water Content 167
4.4.2.3.3. Colour 167
4.4.2.3.4. Texture 167

4.4.2.4. Acrylamide Analysis 168

4.4.2.4.1. Sample Preparation 168
4.4.2.4.2. Extract Purification by Solid Phase Extraction (SPE) 168
4.4.2.4.3. LC-MS/MS Analysis of Acrylamide 168

4.4.2.5. Sensory Evaluation 169
4.4.2.6. Statistical Analysis 170

4.4.3. Results and Discussion 171
4.4.3.1. Quality of Dough and Baked Biscuits 171
4.4.3.1.1. Dough pH 171
4.4.3.1.2. Water Content 171
4.4.3.1.3. Biscuit Colour 173
4.4.3.1.4. Biscuit Texture (hardness) 175
4.4.3.1.5. Acrylamide Content 176
4.4.3.1.6. Principal Component Analysis (PCA) Analysis 178
4.4.3.1.7. Sensory Analysis of Biscuits 179
4.4.3.1.7.1. Hedonic Sensory Analysis 179
4.4.3.1.7.2. CATA Sensory Analysis 180
4.4.4. Conclusion 182
4.4.5. Author Contributions 182
4.4.6. Acknowledgement 183
4.4.7. Appendix A: Preparation of Biscuits 183
4.4.7.1. Dough Preparation 183
4.4.7.2. Baking 184
4.4.8. References 186
4.5. *IN VITRO* STARCH DIGESTIBILITY AND ESTIMATED GLYCAEMIC INDEX (EGI) 189
4.5.1. Resistant (RS) and digestible starch (DS) 189
4.5.2. Degree of starch hydrolysis 189
4.5.3. Estimated glycaemic index (eGI) of biscuits 190
4.5.4. Conclusion 192
4.6. FIBRE-ADDED LAMINATED BISCUITS 194
4.6.1. Physical and functional properties of acorn and raw carob flours 194
4.6.2. Dough texture 197
4.6.3. Fibre-added biscuits characteristics 198
4.6.4. Sensory analysis 201
4.6.4.1. Hedonic liking score 202
4.6.4.2. Just-About-Right (JAR) scales 202
4.6.5. Conclusion 205
4.7. ENERGY CONSUMPTION OF BAKING 207
4.8. SUPPLEMENTARY INFORMATION 208
5. CONCLUSION 211
5.1. GENERAL CONCLUSION 212
5.2. CRITICAL REFLECTIONS ON THE PHD PROJECT 213
5.3. PERSPECTIVES AND FUTURE WORKS 214
6. REFERENCES 215
7. PUBLICATIONS AND COMMUNICATIONS 231
7.1. SEMINARS AND INTERNATIONAL MOBILITY 232
7.2. PUBLICATIONS 232

Abbreviations and symbols of measurement

ABC:	ammonium bicarbonate
SBC:	sodium bicarbonate
SAPP:	sodium acid pyrophosphate
LA:	leavening agent
AA:	acrylamide
FFA:	free fatty acid
LPL:	lysophospholipid
RDS:	rapidly digestible starch
SDS:	slowly digestible starch
RS:	resistant starch
DS:	digestible starch
TR:	total starch
GI:	glycaemic index
eGI:	estimated glycaemic index
TDF:	total dietary fibre
NFCs:	neo-formed contaminants
EFSA:	European Food Safety Authority
CONTAM:	EFSA panel on contaminants in the food chain
OEHHA:	Office of Environmental Health Hazard Assessment
UCD:	Chopin Damage Unit
BI:	browning index
ε_r:	relative complex permittivity
ε_r':	dielectric constant
$\varepsilon_r^{"}$:	dielectric loss factor
ε_0:	dielectric characteristics of a void
$\varepsilon_{r(p)}''$:	loss factor related to the polarisation mechanisms of water molecules
$\varepsilon_{r(\sigma)}''$:	loss factor related to the ionic conduction mechanism
σ:	electrical conductivity
tanδ:	loss tangent (or dissipation factor)
P:	power density absorbed by a material in the microwave cavity in W/m^3

E:	electric field strength in V/m
dp:	penetration depth
f:	frequency of the electromagnetic wave
$m_{gas-free}$:	mass of the degassed (gas-free) dough
$V_{gas-free}$:	volume of gas-free dough
ρ_{dough}:	apparent density (bulk density) of dough
V_{dough}:	total volume of the dough sample
V_{gas}:	volume of entrapped gas
m_{buo}:	mass corresponds to the buoyant force acting on the total volume of the dough sample
V_{gas}:	volume of entrapped gas
ρ_{oil}:	oil density
$\rho_{dough-degassed}$:	gas-free dough density
X_i^v:	volume fraction
ρ_i:	density of ingredient *i*
$\emptyset_{dough}$:	void fraction of gas in dough
$m_{W-freezable}$:	freezable water in g/g biscuit dough
Q_f:	energy of fusion
L_f:	latent heat of fusion in J/g
ρ_{bis}:	apparent density of biscuit
$\rho_{solid-bis}$:	real density of biscuit
m_{baked}:	mass of baked biscuit
$\emptyset_{bis}$:	biscuit porosity
P_{ref}:	reflected power
P_{inc}:	incident power
Q-factor:	quality factor
Q:	energy transferred to the food matrix
m:	mass
C_p:	specific heat of the dough in J/g °C
ΔT:	temperature change
P_{eff}:	effective power
$St_{pr,1}$:	partially hydrolysed starch in the sample in g/L or mg/mL

$C_{glucose}$: concentration of D-glucose in the sample

$\mathrm{St_{pr}}$: partially hydrolysed starch in mg

TS_s: total starch in the sample in mg

$eGI_{s,br}$: estimated GI of the biscuit with white bread as the reference

$eGI_{s,gl}$: estimated GI of the biscuit with glucose as the reference

X_i: mass fraction

C_i: specific heat of ingredient *i*

$C_{p,dough}$: specific heat of the dough

X_{W-WB}: water content of the baked biscuit on a wet basis

$m_{W-dough}$: mass of water in dough

$m_{DM-dough}$: mass of dry matter in dough

X^i_{W-WB}: water content of ingredient *i* on a wet basis

m^0_{W-Bis}: initial mass of the water in the biscuit (or the dough piece)

m^0_{Bis}: initial mass of the biscuit

m^{step1}_{W-Bis}: mass of water in the biscuit at the end of the first step of baking

m^{step1}_{Bis}: mass of biscuit at the end of the first step

$m_{Bis,semi-baked}$: mass of semi-baked biscuit per 1 g of initial dough

m^{step2}_{W-Bis}: mass of water at the end of the second step (in a baked biscuit)

m^{step2}_{Bis}: mass of biscuit at the end of the second step (baked biscuit)

$m_{Bis,baked}$: mass of baked biscuit per 1 g of initial dough

Q_{sens1}: energy required to heat the dough to 105°C (T_1)

L_v: latent heat of water evaporation

Q_1: energy required to evaporate 50% of the water in the dough

Q_2: energy required to transform the semi-baked biscuit into the baked biscuit

Q_{sens2}: energy needed to raise the temperature of the dough from 105°C (T_1) to 140°C (T_2)

Q_{ist}: desorption energy

L_{ist}: heat associated with the desorption process under conditions where the number of adsorbed molecules remains constant

$C_{p,semi-Bis}$: specific heat of the dough after evaporation of half of the water

$C_{p-water}$: specific heat of water

$Q_{total-Bis}$: total energy required for baking the biscuit

List of tables

Table 1.1. Flour constituents and the typical analysis of the flour used for biscuit fabrication (Whiteley, 1971). 29

Table 1.2. Typical specification of flour used for chemically aerated biscuits (D. Manley, 2011). 30

Table 3.1. Biscuit ingredients per 100 g of flour (w.b.). 75

Table 3.2. Experimental mixing conditions for laminated biscuit dough containing LAs at atmospheric pressure. 77

Table 3.3. Experimental mixing conditions for laminated biscuit dough with CO_2 over-pressure mixing. 77

Table 3.4. Experimental plan for baking of laminated biscuits containing LAs. 84

Table 3.5. Power values and specific energy for the baking of laminated and rotary biscuits; (SE: specific energy in MJ/kg dough; PXT: power of the top magnetron; PXB: power of the bottom magnetron; PT: total power; RP: reflected power). 97

Table 3.6. Composition of fibre-added laminated biscuits per 100 g of flour. 109

Table 4.1. Characteristics of Evelia, Paulic, and Enilia flours. 120

Table 4.2. Characteristics of laminated biscuits made with Evelia, Paulic, and Enilia flours. 121

Table 4.3. Void fraction of laminated biscuit dough containing LAs for different mixing conditions. 122

Table 4.4. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at different kneading speeds. 123

Table 4.5. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at different kneading times. 123

Table 4.6. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at 30°C and 25°C. 124

Table 4.7. Laminated biscuit (containing LAs) attributes at various baking conditions. 125

Table 4.8. Properties of LAs-free laminated dough with two CO_2 over-pressure mixing protocols and laminated biscuits. 127

Table 4.9. Laminated biscuit dough recipes; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing). 135

Table 4.10. Biscuit dough mixing protocol. 136

Table 4.11. Dough pieces and biscuit attributes. 142

Table 4.12. Biscuits' colour values and colorimetric distances between control and reformulated biscuits. 143

Table 4.13. Hardness of biscuits. 144

Table 4.14. Dielectric properties of biscuit doughs at 2.45 GHz and 23°C. 148

Table 4.15. Dielectric properties of a cake batter at 2450 MHz and 25°C; adapted from (Alifakı & Şakıyan, 2017). 149

Table 4.16. Microwave efficiency (%) for heating liquid water at an incident microwave power of 40 W. 157

Table 4.17. Energy consumption of the SAIREM microwave oven for baking biscuits. 158

Table 4.18. Formulation of biscuits. 166

Table 4.19. Biscuits' colour values and colorimetric distances between controls and reformulated biscuits.... 173

Table 4.20. Hardness of biscuits; Hardness of biscuits. 176

Table 4.21. Power values and specific energy for baking of laminated and rotary biscuits. 186

Table 4.22. The resistant starch (RS), digestible starch (DS), and total starch (TS) contents of biscuits. 189

Table 4.23. Physical and functional properties of acorn and raw carob flours; (AC: acorn flour; CA: raw carob flour; WHC: water holding capacity; OHC: oil holding capacity; SWC: swelling capacity; values with the same letter in the same column are not significantly different (95% confidence interval). 194

Table 4.24. Flours' colour values and colorimetric distances between the control and acorn and raw carob flours. 196

Table 4.25. Physical characteristics of fibre-added biscuits. 199

Table 4.26. Nutritional composition of fibre-added biscuits. 201

Table 4.27. Molar masses of the different substances involved in LAs. 208

Table 4.28. Moles of LAs in 163.36 g of laminated biscuit dough. 209

Table 4.29. Computed mass of the substances released from LAs during decomposition, along with the corresponding volumes per gram of dough (w.b.), using the ideal gas law at atmospheric pressure and a temperature of 80°C. 209

List of figures

Figure 1.1 Representation of the degree of gluten development in baked products (Cauvain & Young, 2006); the transversal axis shows the decrease in gluten development with an increase in both sugar and fat; (LR cakes: Low-Ratio cakes, traditional-style cakes where sugar is less than or roughly equal to flour by weight; HR cakes: High-Ratio cakes, traditional-style cakes where sugar content is greater than flour by weight)..... 27

Figure 1.2. Biscuit formulas expressed in water, fat, and sugar content (based on 100 of flour); adapted from (Tharrault, 1994). 28

Figure 1.3. Alveograph curve (Dapcevic et al., 2011). 31

Figure 1.4. Starch (amylose and amylopectin) in wheat grain. Starch is located in the starchy endosperm cells. It consists of two populations of granules, large A-granules, which are 5–40 µm in diameter and disc-shaped, and small B-granules, which are <10 µm in diameter and near-spherical, and may be digested at different rates. Adapted from (P. R. Shewry et al., 2020). 32

Figure 1.5. Classifications of wheat protein (Zang et al., 2022). 33

Figure 1.6. Schematic drawing of gluten proteins structure, where gliadins are represented by spheres and glutenins by filaments. The bulkier structure in the upper part shows the gas retained in the gluten network and consequent dough volume expansion observed in the baking process. The slimmer structure in the lower part represents plasticity, extensibility and viscous properties of the gluten matrix (Melim Miguel et al., 2013). 33

Figure 1.7. Dough changes during baking; the changes of the dough piece are involved: 1) heating the proteins and starch to temperatures where swelling, gelatinisation, denaturation and setting come about 2) the release of gases from chemical leaveners 3) these gas bubbles expand as a result of rising temperatures, which also raise the pressure of water vapour inside them 4) some of these bubbles burst and coalesce 5) loss of moisture from the product surface by evaporation, followed by migration of moisture to the surface and continued loss to the oven atmosphere 6) the concentration of the sugar solution increases with temperature 7) a decrease in the consistency of fat and sugar solutions as the temperature rises (D. J. R. Manley, 1998a)..... 42

Figure 1.8. Energy demand of different types of biscuits; CR: cracker; LFS: low fat/sugar; SSB: semi-sweet biscuit; CC: chocolate-coated; CCB: chocolate cream biscuit; VCB: vanilla cream biscuit; RM: raw material; MA: manufacturing; PA: packaging; DC: distribution; RE: retail; CO: consumption; TR: transport; EOL: end of life (Konstantas et al., 2019). 44

Figure 1.9. Heat generation by a) ionic conduction and b) dipole rotation under the action of an electromagnetic field; adapted from (Bou-Orm et al., 2021)..... 48

Figure 1.10. Mechanisms of dietary fibres in the gastrointestinal tract; In the stomach and small intestine, swelling and viscosity of fibres affect transit time, nutrient uptake, and bile acid reuptake. Colonic short chain fatty acids (acetate, propionate, and butyrate) are made through fibre-fermentation by commensal bacteria. Butyrate provides energy for colon epithelial cells and affects cell signaling and immune tolerance, whereas acetate and propionate are taken up into the blood to exert various functions including energy provisions and possibly satiety in the brain satiety center(Carlsen & Pajari, 2023)..... 57

Figure 1.11. Digestion stages and their influence on GI; food digestion involves three important stages: oral phase, stomach phase, and intestine phase (Priyadarshini et al., 2022). 59

Figure 2.1. The scientific approach; (ABC: ammonium bicarbonate; AA: acrylamide). 71

Figure 3.1. Prototype batch mixer VMI (St-Hilaire-de-Loulay, France). 76

Figure 3.2. Air and CO_2 conditions in the VMI mixer at different mixing stages for CO_2 over-pressure mixing procedures, both with and without gas flow. 78

Figure 3.3. Laminated biscuit dough mixing procedure at atmospheric pressure; (LB-ABC: laminated biscuit dough containing LAs mixed at atmospheric pressure; LF-LB: LAs-free laminated biscuit dough mixed at atmospheric pressure). 79

Figure 3.4 Laminated biscuit dough with CO_2 over-pressure mixing (a) with gas flow and (b) without gas flow; (LB-CO2: laminated biscuit dough free from ABC, containing SBC and SAPP 10 with over-pressure CO_2 mixing; LF-LB-CO2: laminated biscuit dough free from LAs with over-pressure CO_2 mixing). 80

Figure 3.5. KENWOOD (France) mixer equipped with a flat K-shape beater. 82

Figure 3.6. RONDO (Germany) dough sheeter. 83

Figure 3.7. Rotary moulding machine (PMP Food machinery, Italy). 83

Figure 3.8. Temperature and humidity data logger HYGROX™-P4 (MCQUEEN CAIRNS, England). 84

Figure 3.9. Mass loss measurement system for biscuits during baking designed at the GEPEA. The oven tray was attached to a balance positioned above the oven, allowing the mass of the biscuits to be recorded during baking. 85

Figure 3.10. CIE L*a*b* colour space. 87

Figure 3.11. Dielectric probe DAK-TL2 (Schmid & Partner Engineering AG, Switzerland) for measuring the dielectric properties of biscuit doughs. 90

Figure 3.12. Modified household microwave oven connected to a 200 W solid-state generator SAIREM (France). 90

Figure 3.13. Vector network analyser S5045 (Copper Mountain Technologies, US) for measuring the electromagnetic properties of materials and systems. 91

Figure 3.14. 200 W solid-state microwave generator (GLS200, SAIREM, France). 92

Figure 3.15. Optical fibres connected to a data logger (Neoptix Reflex®, Canada) placed within a biscuit at the optimal position in the microwave cavity. 93

Figure 3.16. Ramalhos oven equipped with five infrared lamps. 93

Figure 3.17. Placing water in the microwave cavity for evaluating the energy efficiency of the microwave. 94

Figure 3.18. SAIREM prototype microwave oven schema used for industrial baking tests, equipped with 8 magnetrons with a nominal power of 2 kW each. 97

Figure 3.19. Composition of panel used for the hedonic and CATA tests on conventional and microwave-baked laminated and rotary biscuits (n = 69). 101

Figure 3.20. List of CATA sensory analysis attributes. 101

Figure 3.21. Raw carob flour (Creta, Greece) and its nutrition facts per 100 g. 106

Figure 3.22. Acorn flour and its nutrition facts per 100 g. 107

Figure 3.23. Fibre-added laminated biscuits mixing procedure. 110

Figure 3.24. Composition of the panel used for the hedonic and JAR tests on fibre-added biscuits (n = 85). ... 111

Figure 3.25. Steps of biscuit baking in an oven; the biscuit baking process involves two steps: it is assumed that 50% of the water is evaporated during the first step, and the remaining water is removed in the second step to achieve the target final water content of 4% (w.b.). 117

Figure 4.1. Appearance of laminated biscuits (containing LAs) baked under different conditions. 125

Figure 4.2. Oven temperature, dew point (°C), and specific humidity (g /kg humid air) registered by HYGROX for the baking condition of 240-250°C for 9 min and 30 s for laminated biscuits. 126

Figure 4.3. Fusion energy of the frozen water in the dough (J/g) determined by DSC; each curve represents one test. 128

Figure 4.4. Prototype batch mixer VMI. 135

Figure 4.5. pH of biscuit doughs at 25°C;(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval). 140

Figure 4.6. Void fraction of gas of the biscuit doughs; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval). 140

Figure 4.7. Water content of biscuits; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval). 142

Figure 4.8. Biscuits made from the control and reformulated doughs; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing)..... 143

Figure 4.9. S11 value in dB (a) without and (b) with a ceramic resonator at the optimal position for a laminated biscuit; the X-axis represents the microwave frequency in GHz, and the Y-axis represents S11 in dB. 151

Figure 4.10. S11 value in dB (a) without and (b) with a ceramic resonator at the optimal position for a rotary biscuit; the X-axis represents the microwave frequency in GHz, and the Y-axis represents S11 in dB. 152

Figure 4.11. (a) Temperature development of a laminated biscuit in the microwave cavity at a power of 30 W (b) temperature registered at four spots of a laminated biscuit using optical fibres; bars with the same letter at each baking time are not significantly different (95% confidence interval). 153

Figure 4.12. Mass loss of a microwave-baked laminated biscuit at a power of 30 W during 15 min baking. 154

Figure 4.13. (a) Microwave-baked laminated biscuit and (b) combined microwave-infrared baked laminated biscuit. 154

Figure 4.14. (a) Temperature development of a rotary biscuit in the microwave cavity at a power of 30 W (b) temperature registered at four spots of a rotary biscuit using optical fibres; bars with the same letter at each baking time are not significantly different (95% confidence interval). 155

Figure 4.15. Mass loss of a microwave-baked rotary biscuit at a power of 30 W during 10 min baking..... 156

Figure 4.16. (a) Microwave-baked rotary biscuit and (b) combined microwave-infrared baked rotary biscuit. 156

Figure 4.17. Temperature evolution of water in the microwave cavity at an incident microwave power of 40 W. 157

Figure 4.18.Acrylamide formation through the Maillard reaction and lipid oxidation..... 162

Figure 4.19. Biscuits with different formulations baked with different baking methods; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated

biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10). 166

Figure 4.20. List of CATA sensory analysis attributes. 170

Figure 4.21. pH of biscuit dough for different formulations; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval). 171

Figure 4.22. pH of biscuit dough for different formulations; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval). 172

Figure 4.23. Correlation of browning index (BI) with acrylamide (AA) content of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10). 175

Figure 4.24. Acrylamide content of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval). 177

Figure 4.25. Temperature changes during (a) conventional baking and (b) microwave baking (measured for three biscuits, each at one specific location) of biscuits. 177

Figure 4.26. Biplot of PCA analysis of biscuits. 179

Figure 4.27. Hedonic liking score of tested biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval). 180

Figure 4.28. Correspondence analysis biplot of biscuits using 40 sensory attributes identified using CATA. 181

Figure 4.29. SAIREM prototype microwave oven schema used for the industry baking tests. It is equipped with 8 magnetrons with a nominal power of 2 kW each. 185

Figure 4.30. Partially hydrolysed starch over time; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10). 190

Figure 4.31. eGI of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval). 191

Figure 4.32. Acorn and raw carob flours. 197

Figure 4.33. (a) Firmness and (b) deformation of doughs containing 20% and 30% of acorn and raw carob flours; (LB-AC-20: laminated biscuit dough with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit dough with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit dough with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit dough with 30% of wheat flour replaced with raw carob flour; bars with the same letter are not significantly different (95% confidence interval). 198

Figure 4.34. The control and fibre-added biscuits included in the sensory analysis; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour). 201

Figure 4.35. Hedonic liking score of fibre-added biscuits; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour; bars with the same letter are not significantly different (95% confidence interval). 202

Figure 4.36. Just-About-Right (JAR) scale percentages of responses grouped in three levels; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour). 204

Introduction

The production of dry cereal-based products such as biscuits and cookies represents an important industrial sector in France, with 14,282 jobs (Alliance7, 2018), although production has remained stable or is even declining. Three main product groups can be distinguished:

- Laminated products (e.g., casse-croûte BN)
- Rotary-moulded products (e.g., petit beurre)
- Wire-cut products (e.g., cookies)

Other specialties also exist, such as *boudoir*, *à la cuillère*, or *langue de chat* biscuits, which are made from more liquid, aerated batters.

The porous structure of biscuits is mainly due to the presence of leavening agents (LAs). Ammonium bicarbonate (ABC) is often used as an LA and must be declared on the packaging, even though it decomposes during baking, releasing ammonia and CO_2. Unlike sodium bicarbonate (SBC), which must be neutralised with sodium acid pyrophosphate (SAPP)**,** ABC leaves no residues. The latter two ingredients (SBC and SAPP) result in residual sodium and phosphate in the final product. Both bicarbonates (sodium and ammonium) are often used synergistically in dry biscuit formulations. However, in the context of clean-label reformulation, the use of SAPP as a neutralising agent is being reduced.

Among process contaminants, acrylamide (AA) formation is considered one of the most significant food safety concerns. In 2015, the European Food Safety Authority (EFSA), through its Panel on Contaminants in the Food Chain (CONTAM), published a comprehensive risk assessment of AA in food, concluding that AA is a genotoxic carcinogen and that dietary exposure raises health concerns for all age groups. AA is metabolised to glycidamide, which is considered the primary genotoxic metabolite responsible for DNA adduct formation and carcinogenicity (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015).

In 2022, EFSA issued a scientific statement reassessing recent publications concerning the genotoxicity of AA and confirmed that the available evidence supports the conclusions of the 2015 Opinion. EFSA further concluded that the margin of exposure approach remains appropriate for risk characterisation of AA, and that an update of the 2015 scientific opinion was not required (European Food Safety Authority (EFSA) et al., 2022).

In response to these risk assessments, the European Commission adopted Commission Regulation (EU) 2017/2158, which has been applied since April 2018 (European Commission, 2017). This Regulation

establishes mandatory mitigation measures and benchmark levels for specific food categories and requires food business operators to integrate AA risk assessment into their HACCP-based procedures. Food producers must identify processing steps that may promote AA formation and implement appropriate reduction strategies. The benchmark levels established by the regulation include 350 µg/kg for biscuits and wafers, 50 µg/kg for bread, 150 µg/kg for products based on maize, oats, spelt, barley, and rice, and 300 µg/kg for products based on bran, whole grains, wheat, and rye. These benchmark levels are not maximum legal limits, but performance indicators that trigger investigation and corrective measures when exceeded (European Commission, 2017).

This study, linked to the ANR CAPS project "Alternative Baking of Dry Cereal Products", aimed to develop alternative baking methods (biscuit model) to reduce energy consumption and improve product quality in terms of nutritional value and AA contamination, and it was co-financed by the Carnot QUALIMENT (ANR) cluster and Oniris-GEPEA.

"Laminated" (casse-croûte type) and "rotary" (petit beurre type) biscuits were considered with the following objectives: 1) adapt the formulation and process (mixing under CO_2) to reduce or eliminate chemical LAs; 2) develop an alternative baking strategy based on microwaves; 3) evaluate the impact of the baking process on the estimated glycaemic index (eGI) of the biscuits using *in vitro* methods; 4) develop nutritionally improved biscuit formulations - such as fibre-added biscuits through the incorporation of acorn and carob flours - and evaluate the impact of reformulations and processes on consumer acceptance; 5) consider the impact of CO_2 mixing and microwave baking on the AA level; 6) assess the energy consumption of baking.

This study is structured into five chapters to address and investigate the stated objectives:

Chapter 1 provides a review of the literature and the background of the study.

Chapter 2 introduces the context, positioning, and objectives of the study and describes the scientific approach adopted to achieve these objectives.

Chapter 3 outlines the study methodology.

Chapter 4 presents the results from each part of the methodology and includes supplementary information relevant to the study.

Chapter 5 is dedicated to the overall conclusion of the present study.

1. STATE OF THE ART

1.1. Biscuits

This chapter presents an integrated state of the art addressing biscuit formulation, processing conditions, and quality attributes, as well as health-related aspects and innovation strategies. Given the strong interdependence between ingredients, processing parameters and final product quality, these aspects are discussed together to provide a global understanding of product–process–quality relationships, which constitute the foundation of the present work.

1.1.1. Biscuits among other cereal products

Cereal-based baked products come in a wide variety of forms, each differing in ingredient composition and proportions. Wheat flour is the main ingredient in most of these products. The type of dough used—classified as either "hard dough" or "short dough"—depends on several factors, including the quality of the wheat flour (especially its protein content and the molecular characteristics of its gluten-forming proteins, glutenin and gliadin), the dough's water content, and the presence of other ingredients.

When flour proteins are properly hydrated, glutenins (which contribute elasticity) and gliadins (which contribute viscosity) interact to form a viscoelastic gluten network. This network is crucial for products like bread, where a strong, elastic dough structure is needed. However, in doughs containing high levels of hygroscopic (water-absorbing) or hydrophobic (water-repelling) ingredients, such as sugar or fat, or when water content is low, gluten development is limited. This is typical of doughs used for products like biscuits. Among bakery products, the strength of the gluten network is highest in bread, lowest in cakes, and intermediate in biscuits. The transversal axis in Figure 1.1 (Cauvain & Young, 2006) shows the decrease in gluten development with an increase in both sugar and fat. Even within biscuit types, the extent of gluten development can vary depending on the specific formulation and processing conditions.

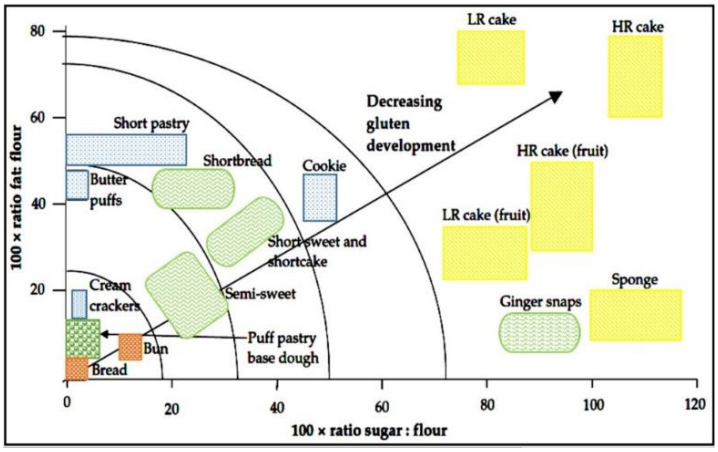

Figure 1.1 Representation of the degree of gluten development in baked products (Cauvain & Young, 2006); the transversal axis shows the decrease in gluten development with an increase in both sugar and fat; (LR cakes: Low-Ratio cakes, traditional-style cakes where sugar is less than or roughly equal to flour by weight; HR cakes: High-Ratio cakes, traditional-style cakes where sugar content is greater than flour by weight).

1.1.2. Classification of biscuits

Biscuits are mainly comprised of wheat flour, sugar, and fat. They are readily available and the most popular baked foods with a long shelf life (Garcia-Armenta et al., 2017). When stored in moisture-proof containers, they have a lengthy shelf life of at least six months with a typical water content in the range of 4% (w.b.). The attraction among consumers is established by the biscuit's appearance and eating qualities (D. J. R. Manley, 1998c).

Traditional biscuits are a sort of flour pastry that may still be prepared and baked in a home kitchen. Nowadays, they are mostly produced in factories located within huge manufacturing facilities. They come in a range of shapes and sizes, and once baked, they can be topped with chocolate, stuffed with a fat filling, or have other delightful garnishes (D. J. R. Manley, 1998a). Given their nutritional value, portability, and extended shelf life, biscuits are popular as a snack food (Dong et al., 2022).

Biscuits are classified in different ways based on the name, the method of forming the dough, and the enrichment of the recipe with fat and sugar (D. Manley, 2001). In order to make dough from wheat flour, water must be added. In fact, by plasticising the wheat proteins, a three-dimensional network is formed that retains the other components. These doughs are typically referred to as "hard" because of their high consistency and a sugar and fat content of less than 20% of the flour content (van der Sman & Renzetti, 2019). They are laminated and then cut into the final shape of the biscuit.

When the proportion of water in a formula becomes low, the amount of fat increases. Doughs are then described as "semi-hard". This general relationship suggests a close link between the functional role of water and that of fat in biscuit-making. Indeed, it is the fat that acts as a binder between the other ingredients and thus contributes to the product's structure (Tharrault, 1994). While this remains partly

solid, the dough paste is poorly structured and forms a more or less coarse sandy paste. It is then the pressure at the forming equipment (rotary press) that creates the product's cohesion.

When the fat content is high, the dough formed is relatively soft and coherent, and its structure is more like an emulsion. In this case, the dough is flexible enough to be shaped with special equipment (wire cutting). The classification of biscuits based on the formula and enrichment of the recipe with fat and sugar is shown in Figure 1.2.

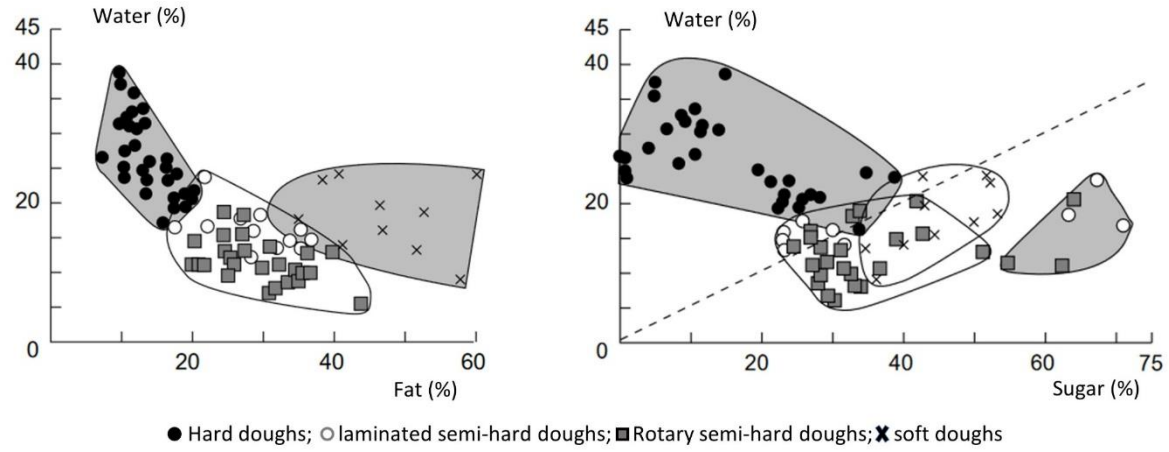

Figure 1.2. Biscuit formulas expressed in water, fat, and sugar content (based on 100 of flour); adapted from (Tharrault, 1994).

Sugar and fat account for a large portion of the biscuit structure. The small amount of water causes a tiny quantity of starch to gelatinise during baking. Gluten, the most abundant of the wheat proteins, has very little effect on the finished texture. However, some gluten must partially develop in the dough. Gelatinised starch and coagulated gluten, along with the oil and sugar content, determine the structure when baked. Sugar and fat soften the gluten, increasing its extensibility and reducing the shrinkage caused by toughening. The presence of fat and sugar softens the gluten, making it more extensible and thereby minimising toughening-induced shrinkage. Many biscuits with a high fat content have a low sugar level in proportion, and vice versa (Whiteley, 1971).

1.1.3. Biscuit sector in France

According to a report published in 2018, the French biscuit industry employs about 12,130 people, representing over 114 production units, and boosts the economy of every region in France. Over 80% of the industry is made up of small and medium-sized businesses (SMEs). The total amount of biscuits consumed per person in 2018 was 5.07 kg. Pastry biscuits, sweet dry biscuits, chocolate and fruit biscuits, cookies, snacks, and breakfast biscuits are all included in this category. In 2018, 78,736 tonnes of biscuits were exported to other countries (Alliance7, 2018).

In 2024, the French biscuit market brought in USD 4,094 million and is projected to grow to USD 5,204 million by 2030. This market is projected to grow at a compound annual growth rate of 4.1% between 2025 and 2030 (Grand view horizon, 2024).

1.1.4. Biscuit constituent

1.1.4.1. Soft wheat flour

The scientific name *Triticum aestivum* L. is a farinaceous grass. Although it is believed to have originated in the Middle East, it is doubtful that the wheat we know today would have looked a thing like those ancient cases. The sort of wheat grown and, ultimately, the flour produced, are strongly influenced by the climate and soil. Additional significant impacts include: Growing region; planting season; grain colour (Whiteley, 1971).

Winter-sown strains often produce soft wheat, which produces soft or weak flour; likewise, white wheats typically produce soft wheat. These are the varieties that are often cultivated in England, and English flour is typically a weak flour that is perfect for making soft-dough biscuits. Yellow wheat often produces flours with medium strength. Red wheats are typically hard as well as spring-sown wheats, both of which yield a tough grain from which hard flour is made. The best wheats for making soft and hard-dough biscuits are probably English and French wheats since they are soft and provide soft flour with pleasant colour and flavour (Whiteley, 1971). Table 1.1 illustrates the flour constituents and the typical analysis of the flour used for biscuit fabrication.

Table 1.1. Flour constituents and the typical analysis of the flour used for biscuit fabrication (Whiteley, 1971).

Flour constituents	Soft doughs and semi-sweet hard doughs
Starch	74.5
Moisture	14.0
Proteins-gluten forming	7.0
Proteins-soluble	1.0
Sugars	2.0
Fat	1.0
Ash (mineral salts)	0.5
	100.0%

A good biscuit flour has the exact opposite properties as a good bread flour. Bread flours, which contain a lot of protein and damaged starch, are made from hard wheat. Biscuits are made with low protein low damaged starch flours. The desire for low-protein, low-damaged starch flour in biscuits becomes evident when one considers the role that protein and damaged starch play as water binders. The goal is to make biscuit dough with a low water content, as the large amount of added water will be removed

during baking. Including substances that bind water makes this goal more difficult to achieve (Edwards 2007). The typical specification of flour used for chemically aerated biscuits is depicted in Table 1.2.

Table 1.2. Typical specification of flour used for chemically aerated biscuits (D. Manley, 2011).

Parameter	Specification	Tolerance
Wheat type	A maximum content of soft wheat varieties	
Moisture content	14.0%	±0.5%
Smell	Free from mustiness (moulds), no taints from paint, detergents, hessian bags, etc.	
Protein (N × 5.7)	9.0%	±0.5%
Colour grade figure (Kent-Jones)	3.5	±1.0
Ash content	0.46–0.55	
Particle size range:		
Particles > 250 µm	Less than 1%	
Particles > 50 µm	40%	±5%
Simon Research values 100E/R	7.5	±1.0
or Alveograph values:		
W (baking strength)	140	±10
P/L or Brabender Extensograph values	1.8	±0.3
Resistance, Brabender units	330	±50
Extensibility, cm	15.6	±1.0

Flour should be a creamy white powder with a mild, pleasant scent, having a slightly sweet taste. It should also tend to stick together when squeezed hard in the hands, but split apart readily as well. It transforms into dough when combined with water that is about half its weight. As previously noted, considering the various types and sources of wheat used in the grist, the composition of flour can vary significantly. Due to the hydrophilic nature of the starch and the storage factors, the water content of flour can range from 11% to 15%. There is a significant risk of bacterial and, more specifically, mold development, if the moisture level rises beyond 14% (w.b.) (Whiteley, 1971).

To assess the quality of dough, alveograph measurements are utilised (Figure 1.3). The force required to inflate and burst a dough bubble is determined by this test. P value, which correlates to the highest height of the curve on the alveogram, indicates the dough's tenacity or resistance to deformation. Lower P values are generally found in flours with weaker gluten. The unit of measurement for this parameter is mm. The L value indicates how extensible the dough is or how much air the dough bubble can contain before popping. L value is demonstrated by the curves length (in mm). The P/L ratio characterises the curves shape and shows how the doughs extensibility and strength (elasticity) are balanced. The dough strength or W value is finally represented by the area under the curve in joules

($\times 10^{-4}$), and it combines the qualities of tenacity (P value) and extensibility (L value) (Bayram & Korkut, 2018).

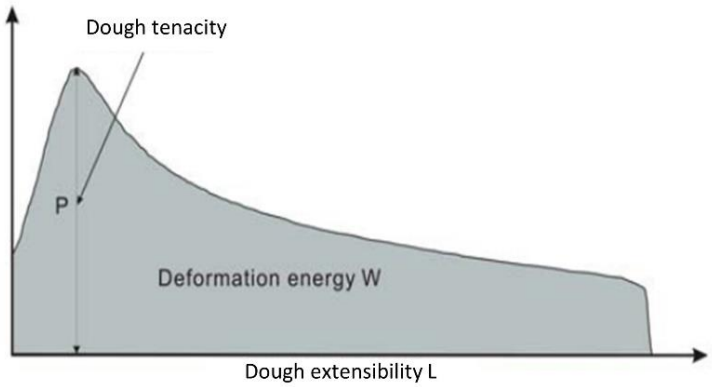

Figure 1.3. Alveograph curve (Dapcevic et al., 2011).

The recommended alveograph values for wheat flour intended for biscuit production correspond to soft flours, characterized by a P/ L ratio between 0.3 and 0.5, and a W value ranging from 80 to 150 (Association nationale de la meunerie française & ARVALIS, 2025).

1.1.4.1.1. Primary components of flour: starch

Wheat flour's primary ingredient is starch. About 98–99% of the dry weight of starch granules is made up of two forms of α-glucans: amylose and amylopectin. The starch plant origin affects the ratio of both polysaccharides. Starch contains major fractions of branched amylopectin connected by α-1,4 and α-1,6 glycosidic bonds and minor fractions of linear amylose connected by α-1,4 glycosidic bonds (Kim & Kim, 2021; Van Rooyen et al., 2023). These two molecules are found in starchy endosperm cells and they have different structures and properties (Figure 1.4). Characteristics of wheat starch are related to the proportion of A- and B-type starch granules. A-type granules are usually disc-shaped, have a diameter of 5 to 40 µm, whereas B-type granules are semi-spherical, and have a diameter of 1 to 10 µm. When starch is heated with water, it can cause irreversible granular swelling, amylose polymer leaching molecular order loss, and granule structure disruption. Thus, the crystal stability determines the starch's gelatinisation temperature (P. R. Shewry et al., 2020). Although the typical starch composition of wheat is composed of ~25% amylose and ~75% amylopectin, genetic approaches that target enzymes involved in starch synthesis have produced waxy wheats, which contain up to 100% amylopectin and high amylose wheats, which have a higher proportion of amylose (Arcangelis et al., 2021).

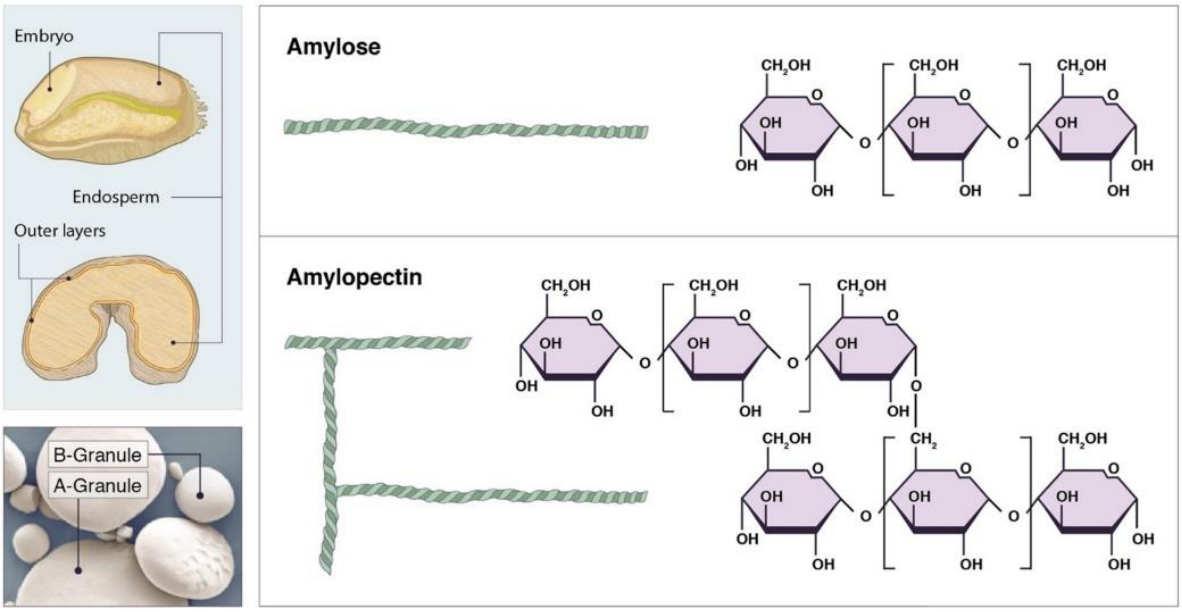

Figure 1.4. Starch (amylose and amylopectin) in wheat grain. Starch is located in the starchy endosperm cells. It consists of two populations of granules, large A-granules, which are 5–40 µm in diameter and disc-shaped, and small B-granules, which are <10 µm in diameter and near-spherical, and may be digested at different rates. Adapted from (P. R. Shewry et al., 2020).

Lipids are integral to cereal starches in the form of both free fatty acids (FFA) and lysophospholipids (LPL), and they have a positive correlation with the amylose fraction. Up to 2% of the weight of starch may be made up of LPL in high amylose cereal starches. Nevertheless, the granules might be impure due to surface lipid contamination. Among the contaminants are triglycerides, glycolipids, phospholipids, and free fatty acids derived from sources other than starch. These lipids are different from lipids that are integral, which are made up of the LPL and FFAs (Tester et al., 2004).

In addition, starches contain comparatively small amounts (< 0.4%) of minerals (calcium, magnesium, phosphorus, potassium, and sodium), all of which have minimal functional significance except for phosphorus. There are three main forms of phosphorus: inorganic phosphates, phospholipids, and phosphate monoesters. Amylopectin molecules have specific regions to which phosphate monoesters are selectively bound (Tester et al., 2004).

1.1.4.1.2. Primary components of flour: protein

The wheat proteins are the ones that are most useful in baking. One characteristic that sets wheat proteins apart is their capacity to form gluten, a viscoelastic mass. Rye is the only other grain with proteins with comparable qualities, although its protein development is less successful (Edwards, 2007). Traditional classifications of wheat grain proteins have been divided into four Osborne categories. Fractions are categorized based on whether they dissolve in aqueous alcohols (gliadins), salt solutions (globulins), water (albumins), or diluted acid or alkaline solutions (glutenins). This

classification provides a way to recover gluten proteins, specifically glutenins and gliadins, in addition to non-gluten proteins, primarily albumins and globulins (Gammoh et al., 2023).

Gluten is a complex mixture, with between 50 and 100 individual proteins being separated by two-dimensional electrophoresis. Gluten comprises about 80% of the protein in wheat flour. These are traditionally separated into two groups: monomeric gliadins, which are traditionally referred to as prolamins, and polymeric glutenins, also known as glutelins (P. Shewry, 2023). As the structural compositions of the proteins glutenin and gliadin differ, they serve different functions during the dough-making process (Figure 1.5). While gliadins are monomers that interact with glutenin polymers through non-covalent forces, particularly hydrogen bonds, glutenins utilize inter-chain disulfide bonds to stabilise their polymers. The polymeric protein network that gives dough its cohesiveness and elasticity is thought to be created by glutenin proteins, while gliadins act as plasticizers of the glutenin network, giving dough its viscosity and extensibility (Zang et al., 2022).

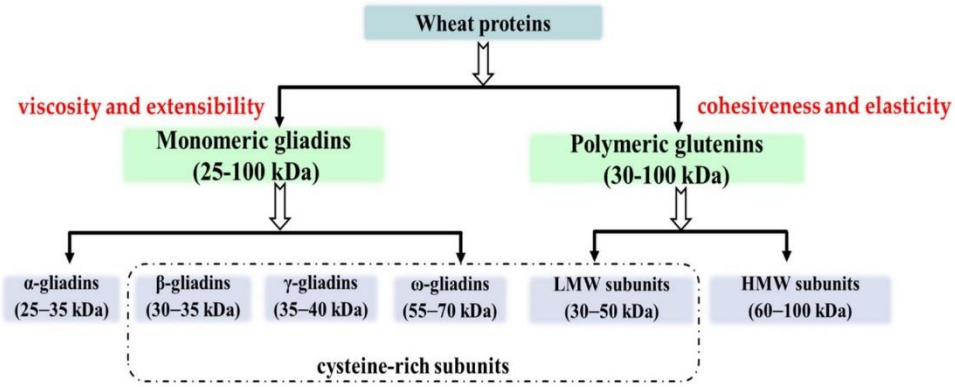

Figure 1.5. Classifications of wheat protein (Zang et al., 2022).

Gluten proteins create a continuous three-dimensional network (Figure 1.6) when wheat flour and water are combined to make dough, and both covalent and noncovalent forces stabilise this network. Interchain disulfide bonds that are covalent stabilise the glutenin polymers (P. Shewry, 2023).

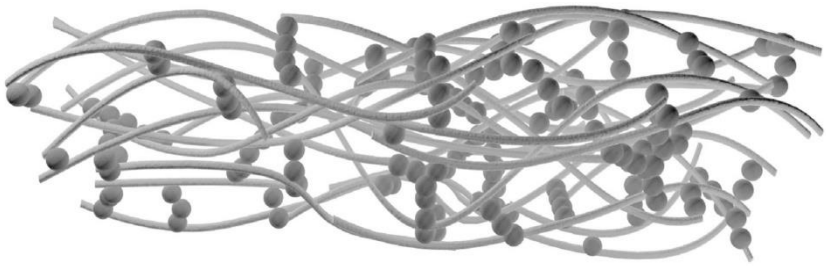

Figure 1.6. Schematic drawing of gluten proteins structure, where gliadins are represented by spheres and glutenins by filaments. The bulkier structure in the upper part shows the gas retained in the gluten network and consequent dough volume expansion observed in the baking process. The slimmer structure in the lower part represents plasticity, extensibility and viscous properties of the gluten matrix (Melim Miguel et al., 2013).

It is believed that the protein content of flour has a direct impact on biscuit quality. The biscuits with higher protein and gluten will gradually get harder. Soft wheat flour of high quality yields large spread cookies that are low in thickness and have a large diameter. The gluten network is crucial for bread and other soft products, but it is not essential for biscuits. In reality, a cohesive but not particularly elastic dough requires only a slight development of the gluten network. It's also said that gluten can be omitted from biscuit recipes. To satisfy the needs of those with celiac disease, biscuits of a respectable textural quality can be produced using flours from a variety of gluten-free grains such as sorghum, pseudo cereals, and legumes. Therefore, gluten might not be as important to the production and final quality of biscuits (Liu et al., 2022).

1.1.4.2. Sugar

Apart from imparting sweetness, sugar serves as a key ingredient that influences the structure and flavour enhancement of baked goods. The dissolution of sugar in biscuit dough, whether complete or partial, is determined by the water content available. Following baking, the recrystallisation of sugar crystals contributes to the creation of a crispy and crunchy texture in the biscuit. The size of the sugar crystals and their dissolution rate play a crucial role in determining the level of crunchiness in the final product. Biscuits made with coarser sugar crystals tend to exhibit less dough spread and more surface cracking. The quantity of sugar utilised significantly impacts the sensory characteristics of the biscuit, affecting its hardness and visual appeal. An excess amount of sugar can result in a softer dough with reduced consistency and cohesion. Sugar with a high concentration and fine grain size facilitates substantial spreading during the baking process (Arepally et al., 2020).

Short-dough biscuits have the most sugar, semi-sweet biscuits have less, and crackers have the least. Short doughs have very little water, which prevents the sucrose from dissolving. Since the sugar will be present in solid form, the crystal size is significant. A hard, glassy biscuit can arise from a high sucrose content in short dough, most likely because the molten sucrose cools to create a glass. Such a biscuit will get softer if glucose syrup is included. All of the existing reducing sugars are capable of undergoing the Maillard reaction, which results in appealing colour and flavour. Dextrose, fructose, maltose, and lactose are all reducing sugars among the potential sugars available. Sucrose is the only non-reducing sugar that is most likely to be present. Sucrose, often in the form of a solid and glucose syrup, is the typical sugar constituent in biscuits. Skimmed milk, whey powder, and lactose can all be sources of lactose. Malt extract, which contains maltose and contributes flavour and colour, is another product that contains sugar. Sugars can be added to a product using malt extract without being identified as such on the label (Edwards, 2007).

1.1.4.3. Fat

The primary technical definition of the phrases "fat" and "oil" is to describe the physical condition of a fat; they are only vague descriptive terms. A lipid is often referred to as a fat if it is solid at 15°C, and an oil if it is liquid at that temperature. The three types of fats and oils that make up nature's fats and oils are mineral, animal, and vegetable (Whiteley, 1971). Fat is a primary element in bakery products and plays a vital role in both the dough and the final biscuit. The quantity and type of fat integrated into the dough significantly influence its viscoelastic and rheological properties (Kouhsari et al., 2022). Fats play a crucial role in dough by acting as a shortening agent. Shortening, in this context, refers to the ability of fats to lubricate, weaken, or shorten the structure of food components to achieve the desired texture in the final product (Kouhsari et al., 2022). During mixing, fats act as a lubricant and prevent the formation of a gluten network in the dough (Mamat & Hill, 2014). Without shortening, the water or sugar solution would interact with the flour protein to form cohesive and stretchy gluten. However, with the presence of fats, they surround the proteins and starch granules, isolating them from the water and disrupting the continuity of the protein and starch structure (Mamat & Hill, 2014).

The majority of biscuit varieties have a significant fat content. Butter is the first biscuit fat, and some biscuits are made entirely of butter. In this situation, butter gives the goods flavour and an advantage in marketing. Any fat used in biscuits must be stable during storage because they are a long-lasting product (Edwards, 2007).

1.1.4.4. Water

Water—both added directly and present in ingredients such as butter and flour—is almost entirely evaporated during baking. Therefore, it can be considered more of a processing aid or catalyst than a final ingredient. Despite its near-complete disappearance from the finished biscuit, water plays a crucial role during production, especially in the mixing stage. It facilitates the dispersion and hydration of ingredients like flour and LAs, and it also helps dissolve certain components (Cauvain & Young, 2006). The limited amount of water used in biscuit formulations, along with its restricted availability to proteins and starch, contributes significantly to the characteristic crispiness of biscuits (Misra & Tiwari, 2014). However, increasing the water content in the recipe can reduce dough consistency, making it more fluid and sticky (Maache-Rezzoug et al., 1998a).

1.1.4.5. Leavening agents (LAs)

Aeration must be achieved in some way in order to enhance texture, bite, appearance, and taste. Aeration techniques may be generally categorised into three categories: mechanical, biological, and chemical. The employment of chemicals that may produce a gas but leave no disagreeable flavours or

odours or adverse consequences is by far the most common and significant form of aeration. ABC (NH_4HCO_3) and SBC ($NaHCO_3$) are the most significant aerating agents. Without the use of additives, mechanical aeration is accomplished by manipulating the product in a certain way. The two basic techniques are beating and mixing, in which air is added to the product and continuously divided into extremely small bubbles by the beater or mixer's blades. To trap these air bubbles and prevent them from escaping during baking, the product must contain some substance. An egg, albumen, or gelatin-type protein is typically responsible for this (Whiteley, 1971), even though it is not used in all recipes. The addition of LAs to dough is essential for the production of leavening gases, which contribute to the desired texture of biscuits when they are baked (Arepally et al., 2020).

SBC is the most widely used chemical LA in baking due to its ability to release CO_2. When heated, CO_2 and water are released, leaving sodium carbonate as the residual salt (*Eq. 1.1*). Sodium carbonate is a compound with undesirable sensory effects such as soapy taste (especially in the presence of rancid fats) and darkened crumb colour, and it has a gluten-softening effect that increases product spread. To improve its performance and minimise negative effects, SBC is typically partially neutralised with acid ingredients, such as SAPP, in biscuit formulations *(Eq. 1.1*). This acid–alkali reaction enhances CO_2 release without fully eliminating the softening effect of the remaining sodium carbonate, which can be beneficial for spread and texture.

$$NaHCO_3 + H^+ \rightarrow Na^+ + H_2CO_3 \qquad \textit{Eq. 1.1}$$

$$H_2CO_3 \rightarrow CO_2 + H_2O \qquad \textit{Eq. 1.2}$$

For effective aeration, the acid must be insoluble in cold water but become active at oven temperatures, ensuring that gas release occurs during baking rather than during dough preparation. SBC, although technically an acid salt, behaves as an alkali in this context and is soluble in cold water, necessitating careful selection of a thermally-activated acid to optimise leavening while minimising flavour and colour defects (D. Manley, 2011).

ABC is a highly effective LA in biscuit production due to its complete thermal decomposition into CO_2, ammonia (NH_3), and water when heated (around 60°C and above) (*Eq. 1.3*). It is also less expensive than SBC. This decomposition contributes significantly to dough aeration without leaving alkaline residues, which makes it suitable for applications where a neutral final pH is desirable. This LA has a strong ammonia odour in solid form, is highly water-soluble, and is strongly alkaline, resulting in softer doughs with reduced water requirements for the same consistency. Despite its volatility, the gas loss is minimal at room temperature, even after prolonged exposure in solution (D. Manley, 2011).

$$NH_4HCO_3 \rightarrow NH_3 + CO_2 + H_2O \qquad \textit{Eq. 1.3}$$

ABC strong alkalinity promotes increased dough spread, which can be beneficial for short dough formulations. However, care must be taken in handling: it is prone to lumping, even in dry storage, and should be used quickly after receipt, preferably pre-dissolved in water before mixing. If pH adjustment is needed in the final baked product, SBC may be added separately (D. Manley, 2011).

1.1.5. Minor ingredients

1.1.5.1. Glucose syrup

A mixture of sugars, including glucose, maltose, and other nutrient-dense saccharides, is produced by hydrolysing edible starch and used to make glucose syrup (Teshome et al., 2025). The role of glucose syrups in biscuits is quite defined. They help improve surface coloration through the Maillard reaction and create a crispy texture in savoury biscuits without overwhelming sweetness. Moreover, these syrups are practical and economical for regulating moisture distribution, sweetness levels, and sucrose crystallization in sugar confectionery and jams (D. Manley, 2011).

1.1.5.2. Salt

A mineral compound called sodium chloride (common salt) is necessary for health and vitality in tiny amounts. Salt is equally important to our taste while consuming the majority of foods. Salt-free biscuits are uninteresting rather than flavourless. While there is flavour in the biscuit, it is barely noticeable and remains in the background without salt. The flavour is enhanced, amplified, and drawn out by the salt. Depending on the kind of mixture and the presence of additives with strong flavours, different amounts of salt are needed, although typically around 1% of the weight of the flour is used. It is important to consider the salt content of other substances. For instance, if butter or margarine is used in the mixing, the salt quantity must be reduced to reflect the salt content of the fat used (Whiteley, 1971). Salt is used to decorate and dress the surface of savoury biscuits. Furthermore, it toughens the gluten, increases the consistency, and hinders the fermentation and the Maillard reaction (Arepally et al., 2020).

1.1.5.3. Dairy ingredients

Skimmed milk powders are used in the production of biscuits rather than liquid milk, which is rarely employed due to practical concerns about stability. To ensure that it is distributed uniformly throughout the end product, this is often dissolved in water that is twice its weight. It is necessary to refrigerate the reconstituted milk powder since it has the same preservation qualities as liquid milk. A

completed product with only a dry blend of the milk powder is probably going to have little brown caramelised milk powder particles in it. Protein and lactose, two key ingredients in milk powder, are both beneficial. The Maillard reaction transforms lactose, a reducing sugar, into flavour and colour. These advantages can only be achieved if the proteins are dissolved or dispersed alongside the lactose (Edwards, 2007).

1.1.5.4. L-Cysteine

L-Cysteine is an amino acid that serves as a building block of some proteins. It is of the most often used reducing agents in baking. The addition of this reducing agent decreases the stability and water absorption capacity of both weak and medium-strong wheat flour. The percentage of wet and dry gluten recovery decreases as a result of reducing agents interfering with the formation of the gluten network. Reducing agents were found to decrease dough strength while increasing dough extensibility. The addition of reducing agents increases the cookies' spread factor while decreasing their hardness. Therefore, it can be said that reducing agents are helpful in making dough more extensible and decreasing its strength (KUMAR et al., 2013).

1.2. Fabrication of biscuits

The manufacturing stages in a cereal product are the formation of the dough through mixing and baking, which transforms the dough into an expanded final solid product. Biscuit ingredients are first mixed in one or two stages for different durations. The resulting dough is then shaped into biscuits using various methods, and the dough pieces are placed in the oven. Once baked, the biscuits are cooled and packed. This manufacturing process may include additional steps depending on the type of product.

1.2.1. Mixing

Mixing influences the development of gluten and, consequently, the rheological properties of the dough, as well as the homogeneous distribution of the various ingredients. It is therefore very important for the quality of the finished product (Jia et al., 2020). There are two fundamental ways to mix biscuit doughs (Edwards, 2007; Whiteley, 1971); however, each may be modified for best results based on the equipment available and the specific conditions present. For soft dough biscuits, the cohesion of the dough observed during pressing in a mould is mainly due to the presence of fat, which acts as a plasticiser.

"Creaming method" is the mixing technique mainly used for short dough biscuits. To create a smooth, homogenous cream, the fat and sugars, including syrups and other common ingredients, are blended. This stage introduces air bubbles into the mixture, which remain trapped in the liquid fat phase (van der Sman & Renzetti, 2019). During mixing, some of the sugar dissolves in the water, forming a syrup that blends with the fat. At the same time, the undissolved sugar particles rub against the solid fat, breaking it into smaller pieces and increasing the number of fat crystals. This helps the fat trap and stabilise air bubbles more effectively, improving aeration of the (Miller, 2016). The addition of colours and essences occurs at this stage. The addition of colours and essences before the components have fully blended is not advisable since there is a chance that one of the ingredients will absorb liquid colour, leaving marks of colour in the mixture. The aerating agents and salt are dissolved in some of the dough water while the cream is being made. These are blended in with the cream for a short duration of time after being introduced together with reconstituted milk. Any residual water is added once the flour begins to mix with the cream after being placed into the mixer. The dough is mixed continuously until it has sufficiently evolved to fit the particular biscuit-shaping equipment for which it is designed (Whiteley, 1971).

The second way is referred to as the "all-in method", in which all of the components are placed into the mixer before the dissolved aerating agents, salt, colour and essence, and water are added as the process of blending gets started. Although this procedure is simpler and more direct than the creaming method, the dough it tends to generate is somewhat denser and tougher. Semi-sweet hard doughs are mixed with the "all-in method". LAs and dissolved salt are added to the flour and other ingredients, and the dough is then stirred until it develops as for a fermented dough using an all-in approach. However, mixing continues until the mechanical development softens the gluten, and finally, the elasticity of the gluten is lost, and it becomes extensible (Whiteley, 1971).

1.2.2. Forming

Biscuits are formed according to the type of dough.

Laminating: The equivalent of a domestic rolling pin is sheeting. To make dough with a specific thickness, the dough is fed between rollers from a hopper. The issue with this method is that the dough is worked on while rolling, which results in the development of gluten. By employing just one pair of rollers, this issue may be minimized. Gauge rollers are the rollers that measure the thickness of the dough. The dough pieces will shrink while cooking if the dough is delivered to the cutter under strain, and they will often emerge thicker at the front and backside. Allowing the dough to rest before cutting is the solution to this issue. In order to achieve their unique texture, semi-sweet hard doughs are typically laminated to create layers. Laminating can be done manually or using an automated laminator (Edwards, 2007).

Extruding Wire Cutting and Depositing: Biscuits may be shaped most easily by extruding them through a die. If the dough is extremely soft, it might be deposited like a batter. Wire cutting is an option if the dough is firm. The pieces are cut when the dough is driven through a die, and a tight wire placed in a frame passes across the die. As extrusion continues, the wire is repositioned away from the die for the reverse stroke. A straight or serrated knife blade can be used in place of the wire, which can either be vibrated while it cuts. Sticky doughs and doughs with additional ingredients, such as almonds or oat flakes, that could not be rotary moulded, can be handled by wire cutting (Edwards, 2007).

Rotary Moulding: Particularly soft dough biscuits can be shaped with rotary moulding machinery. The main components of rotary moulders are the moulding roller, a forcing roller with grooves, and an extraction roller covered with rubber. The forcing roller forces dough from the hopper into a set of biscuit molds that are formed on the cast bronze face of the moulding roller. A steel scraper is used to remove extra dough from the moulding roller (Whiteley, 1971).

1.2.3. Baking /cooling

1.2.3.1. Conventional baking

After shaping, the biscuits are baked for a short time (a few minutes) at high temperatures, usually between 180°C and 240°C (Misra & Tiwari, 2014). Baking is a type of cooking done in an oven, and it's a complicated process that involves mass and heat transfer. Under the influence of heat, it turns semi-solid dough into a consumable product. The dough pieces frequently spread during baking, contingent on the oven conditions, dough preparation and recipe. The biscuit's structure and texture are first formed by the removal of moisture, and then it browns in the last stage. Knowledge of heat and mass transfer is necessary to keep the baked biscuits uniform in colour, texture and desired final water content. All three forms of heat transfer are important in the making of biscuits. Radiation, convection, and conduction all play a part in the browning reaction, moisture removal and dough expansion, respectively (Arepally et al., 2020).

Protein denaturation, starch gelatinisation, and non-enzymatic browning contribute to the distinctive final product structure in the baking mechanism. Three main stages can be distinguished in baking: dough expansion, surface desiccation, and crust browning. Temperature increases during baking control these phases: the doughs quick volume expansion (5–10 min) at 35–70°C, the elimination of yeast and other contaminating microorganisms and the termination of CO_2 formation at 60–70°C, the gelatinisation of starch at 76°C, the inactivation of enzymes at 80–95°C, and finally the formation of the crust at 105°C followed by browning at 160°C (Devu et al., 2022). All of these changes are caused by temperature and involve various parts of the recipe and the formed dough piece (Figure 1.7).

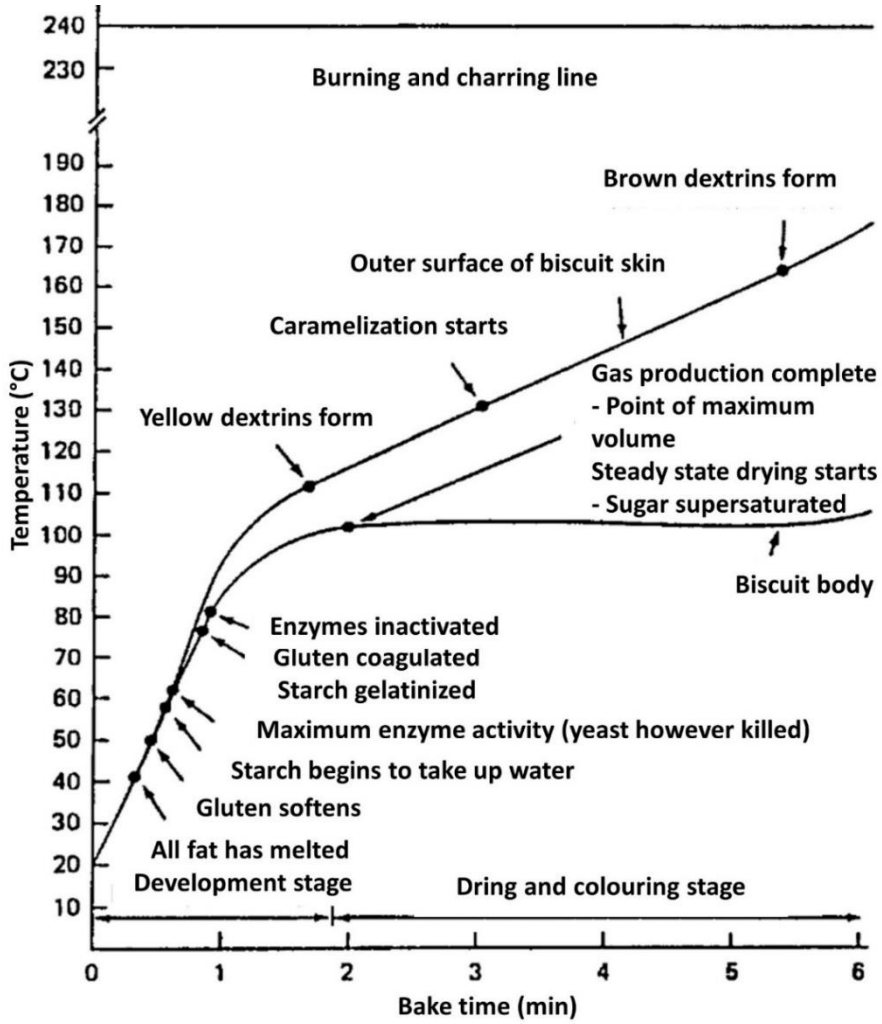

Figure 1.7. Dough changes during baking; the changes of the dough piece are involved: 1) heating the proteins and starch to temperatures where swelling, gelatinisation, denaturation and setting come about 2) the release of gases from chemical leaveners 3) these gas bubbles expand as a result of rising temperatures, which also raise the pressure of water vapour inside them 4) some of these bubbles burst and coalesce 5) loss of moisture from the product surface by evaporation, followed by migration of moisture to the surface and continued loss to the oven atmosphere 6) the concentration of the sugar solution increases with temperature 7) a decrease in the consistency of fat and sugar solutions as the temperature rises (D. J. R. Manley, 1998a).

In addition to technological aspects, the baking step represents a major contributor to energy consumption in biscuit manufacturing.

1.2.3.2. Energy consumption and environmental impact of baking

The food industry, the government, and civil society have all made enhancing the sustainability of food production and consumption a top aim over the past few decades. However, the transition to a food system that is healthy, rich in nutrients, and ecologically sustainable faces enormous obstacles as a result of the diversity and complexity of the food system, from the local to the global (Miah et al., 2018). A major product category in the global confectionery market is biscuits. Despite this, their effects on the ecosystem are not well studied (Konstantas et al., 2019).

The confectionery Industry is a very broad and challenging sector. Due to the variety of products, there are complex supply chains, ingredients that are grown in particular parts of the world, specialised equipment to process and transform ingredients, various formulation sciences to create nourishing and delicious recipes, and a variety of retailers with various sales strategies. However, the ingredients and packaging, composition, and pre-processing steps are the main variations amongst confectionery goods(Miah et al., 2018).

Miah et al. (2018) studied the life cycle analysis for five different confectionery products: dark chocolate, milk chocolate, sugar, milk chocolate biscuit, and milk-based products. They found that sugar confectionery had the lowest influence on the environment, whereas dark chocolate confectionery had the most. The milk chocolate confectionery comes in second, followed by milk-based confectionery and milk chocolate biscuit confectionery, in that order. In comparison to sugar-based confectionery, dark chocolate confectionery has a larger than 395% potential to contribute to global warming. The difference between confectionery items with the highest and lowest environmental impacts can be mostly attributed to the raw ingredients. Additionally, they revealed that, among the five confectionery items, the manufacturing stage had the second-highest environmental effect. The energy required for the various processing technologies is mainly responsible for this. Additional research indicated that, on average, for this particular case study, direct energy (such as natural gas) accounts for 66% of energy usage, while indirect energy (such as electricity) accounts for 34%. As a result, there are possibilities to lower the demand for energy.

Konstantas et al. (2019) proved that among all biscuit variations, chocolate-coated biscuits had the highest primary energy demand (22.4 MJ/ kg), followed by the two cream varieties, with consumption levels that are around 14% lower (19.1-19.4 MJ/ kg). The least amount of primary energy is needed by low-fat/sugar biscuits, evaluated at 16.9 MJ/ kg. The two biggest users are raw materials (26% - 47%) and biscuit manufacturing (31% - 47%) (Figure 1.8).

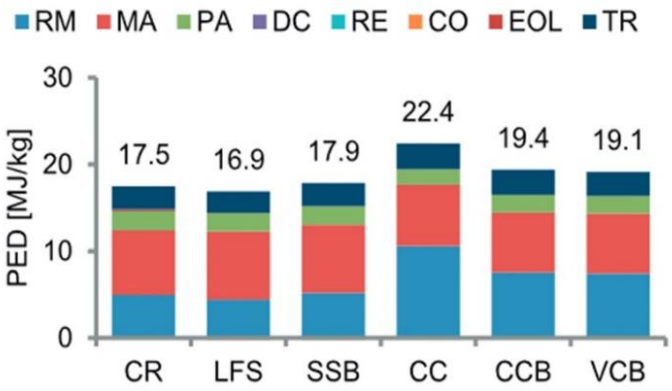

Figure 1.8. Energy demand of different types of biscuits; CR: cracker; LFS: low fat/sugar; SSB: semi-sweet biscuit; CC: chocolate-coated; CCB: chocolate cream biscuit; VCB: vanilla cream biscuit; RM: raw material; MA: manufacturing; PA: packaging; DC: distribution; RE: retail; CO: consumption; TR: transport; EOL: end of life (Konstantas et al., 2019).

Due to the high energy demand associated with conventional baking, alternative heating technologies have been investigated.

1.2.3.3. Alternative baking technologies

The baking step is one of the unit operations that uses the most energy when making biscuits. The large commercial bakeries use less energy per unit mass of the product than small bakeries (Arepally et al., 2020). With an energy consumption of up to 6 MJ/kg for bread dough and less than 40% of the energy specifically used for dough baking and bread water loss, conventional baking is the most energy-intensive method of baking bread. As a result, auditing energy consumption can greatly lower production costs. This high energy consumption raises production costs. Given a minimal investment in time, equipment, or additional labour costs, the bakery industry must meet consumer demand. Ongoing advancements in baking technology are worthwhile primarily for the creation of higher-quality and more nutrient-dense products, as well as lower production costs (Bou-Orm et al., 2021).

Globally, the use of microwave heating for food preparation is continually rising. The major benefits of using microwave heating include much quicker heating, greater energy efficiency, and shorter processing times. Nevertheless, there are still some issues with the procedure. These are in regards to food quality and uneven food heating. Therefore, research has concentrated on ways to solve these issues. In order to produce an effective microwave-material interaction, research is now focused on novel food composition, altering oven design, and combining microwaves with other heating techniques (Ahmed & Ramaswamy, 2007).

To identify the variables and processes influencing checking, theoretical modelling and experimental validation of microwave post-baking versus conventional baking of biscuits were investigated by

Ahmad et al. (2001). Visible checking decreased from an average of 61% to 5% among biscuits that were microwave-baked (at 700 W for 30 s immediately after conventional baking) according to the results. The development of more consistent moisture profiles while keeping the initial biscuit temperature above 110°C during microwaving was determined to be the primary factor increasing mechanical strength and decreasing checking. Once exposed to high ambient humidity, microwave-baked biscuits were also found to be less prone to checking than conventionally-baked biscuits. In comparison to conventionally-baked biscuits, microwave-baked biscuits exhibited a significantly more uniform internal moisture distribution and a faster rate of drying according to heat and mass transfer simulations (Ahmad et al., 2001).

A study was conducted by Demirekler et al. (2004) to compare the quality of bread baked in a halogen lamp–microwave combination oven (with a microwave power of 706 W and 2 halogen lamps) with bread baked in a traditional oven. Microwave power, baking time, and upper and lower halogen lamp power were all independent variables. The bread's weight loss, colour, specific volume, porosity and texture profile were the quality parameters that were measured. The response surface methodology was applied for the optimisation. Most of the quality parameters were found to be significantly impacted by baking time, microwave power and upper halogen lamp power. The lower halogen lamp power, however, was determined to be a negligible factor. 5 min of baking at 70% upper halogen lamp power, 50% lower halogen lamp power, and 20% microwave power were found to be the ideal baking conditions in the halogen lamp–microwave combination oven. Breads baked under ideal conditions were just as good as those baked conventionally. Bread baking times were reduced by 60% when a halogen lamp and microwave combination oven was used (Demirekler et al., 2004).

A comparison was made between the quality of cakes baked in microwave, IR, and IR-microwave combination oven by Sumnu et al. (2005) . Weight loss, specific volume, firmness, and cake colour were the quality parameters that were assessed. Cakes that were traditionally baked were used as a control. The worst-quality cakes were those made in a microwave oven. Through IR and IR-microwave combination baking cake surfaces could develop colour. Cakes made in an IR-microwave combination oven had firmness and colour values comparable to those made in a traditional oven. The ideal settings for cake baking in an IR-microwave combination oven were 50% microwave power and 70% halogen lamp for 5 min. Conventional cake baking times were reduced by approximately 75% when an IR-microwave combination oven was used under these conditions (Sumnu et al., 2005). In a different study by Sevimli et al. (Sevimli et al., 2005) , it was discovered that the baking time, microwave power and upper halogen lamp power had a significant impact on the cakes' firmness, specific volume and weight loss. The baking time and the power of the upper halogen lamp had a major impact on the cake's colour. All quality parameters were found to be unaffected by the lower halogen lamp power

except for weight loss. Using the halogen lamp–microwave combination oven allowed for the production of cakes with quality comparable to cakes baked in a traditional oven. Furthermore, the cake's baking time was greatly shortened. For cake baking, a halogen lamp–microwave combination oven can be suggested (Sevimli et al., 2005).

The baking of various cakes in microwave and near-infrared microwave combination ovens to consider the impact of different formulations on the colour and texture of cakes was examined by Ozge et al. (2006). Cakes were also baked in conventional ovens for comparison. In all baking types, colour and hardness were found to be formulation-dependent. It was discovered that cakes baked in microwave and near-infrared microwave combination ovens with malto dextrin, a fat substitute made primarily of whey protein, were the firmest (Ozge et al., 2006).

In a study conducted by Dong et al. (Dong et al., 2022), biscuits were baked in microwaves at 440–520 W for 2-3 min as opposed to 7 min at 190°C in a traditional oven. Maillard reaction hazards (AA, 5-hydroxymethylfurfural, methylglyoxal, and 3-deoxyglucosone) increased with microwave power and duration. Biscuits had a nice texture and up to 60% less risk than traditional baking when baked at 440 W for 3 min. Stronger browning caused hardness to increase and colour to darken with increasing power and time.

It has been demonstrated that when compared to traditional methods, microwave baking greatly reduces baking time while maintaining a satisfactory level of baked food quality. According to studies, when conditions are right, microwaves can decrease hazardous Maillard reaction products, increase moisture uniformity, and minimise. On the other hand, browning and hardness are increased by higher baking time or power. Despite these benefits, it is difficult to quantify the energy usage and effectiveness of microwave baking because most studies concentrate on product quality rather than precise energy measurements or process modeling.

1.2.3.3.1. Microwave baking of biscuits

Volumetric origin, quick temperature rise, controlled heat deposition, and ease of clean-up make microwave heating appealing for food preparation. It is currently employed in many industrial and domestic food processing and preparation applications. The technology has been used for ready-to-eat meals, pasta-based products, and a wide range of other foods throughout Europe, Japan, and South America. In Italy, microwave processing for fresh-filled pasta has become widespread since the 1990s (Ahmed & Ramaswamy, 2007).

Since the 1940s, microwaves have been employed as a heat source. It began as a development of the radar technology established during World War II. Given their quick cooking, microwaves can process

food more quickly, resulting in fewer alterations to their nutritional and sensory qualities. In contrast to its vital role in domestic consumption, microwave energy has not yet been fully utilised in industrial applications. One of the likely causes is the presence of hot spots in various areas of a processed food that rely on the geometry of the item. Additionally, the limitation includes the issues with microbiological safety and overheating brought by uneven heating during processing. Recent research on the temperature distribution in food materials has provided some suggestions on how to lessen non-uniformity during microwave baking, though. Reformulating food ingredients, designing food packaging, and modifying oven designs to include phase control heating, variable frequency ovens, and pulsed microwave heating are all contemporary trends that have been suggested. The mixing of microwave heating with other heating techniques, such as infrared or forced-air heating, has been started (Ahmed & Ramaswamy, 2007).

In conventional baking, heat is transferred to the product surface mainly through convection from the hot air and radiation from the oven walls, followed by conduction toward the product's centre. The key factors influencing final product quality include the rate and extent of heat transfer**,** relative humidity in the oven, and baking time (Yolacaner et al., 2017).

To better understand microwave baking behaviour, the physical principles of microwave heating are briefly described below:

1.2.3.3.2. Fundamentals of microwave heating

Electromagnetic waves with frequencies between 300 MHz and 300 GHz are known as microwaves. In a dielectric material, microwave heating is the term used to describe dielectric heating brought on by polarisation effects at a particular frequency band. Applications of microwave heating for industrial, scientific, and medical purposes have been restricted to a few specific frequency bands in order to prevent interference with radio frequencies used for telecommunication. The most common microwave frequencies for dielectric heating are 2450 MHz and 915 MHz, and depending on the moisture content, the penetration depths vary (Ahmed & Ramaswamy, 2007). The microwave generator (magnetron or solid-state microwave power amplifiers), waveguides, and a cavity for batch operation or a tunnel with a conveyor belt for continuous operation are some of the fundamental components of microwave equipment, regardless of their designs (Ahmed & Ramaswamy, 2007).

In general, there are three qualitative ways to classify materials based on their microwave energy absorption: 1) absorbers or materials with high dielectric losses that heat up easily under microwaves; 2) transparent or low dielectric loss materials in which microwaves pass through the material with little or no attenuation; 3) solid or conductive materials that reflect microwaves at their surface (Curet, 2019).

In a microwave cavity, food materials heated by microwave undergo volumetric heating primarily due to ionic interaction and dipole rotation, which depends on the microwave frequency (Figure 1.9) (Bou-Orm et al., 2021). Heat is generated inside the food and transferred through the food material as a result of microwave interaction with polar molecules and charged particles. Due to the microwave oven's quick heat generation, heating occurs very quickly. As a result, starch gelatinisation, enzyme conversion, dough/batter expansion, crust formation, browning, and the final setting of the dough/batter into a rigid crumb structure during microwave baking may not be completed (Yolacaner et al., 2017). Both temperature and baking time affect kinetic reactions such as the browning and starch gelatinisation processes. For this reason, when baking time is shortened, it is important to make sure that the gelatinisation and browning reactions are finished, or else the product's quality may be degraded (Yolacaner et al., 2017). The majority of food products contain water as a key ingredient. This is the primary element that interacts with microwaves due to its strong dipole moment, which is caused by the presence of negatively charged oxygen molecules and positively charged hydrogen atoms, mainly at 2450MHz (Bou-Orm et al., 2021). Anwar et al. (Anwar et al., 2015) stated that an ionic conduction effect can result from the interaction of solubilised ions, like sodium chloride, with microwave heating. In their study, water samples with and without inorganic ions were subjected to the same microwave exposure conditions at 2450 MHz in a domestic oven. Comparing pure or diluted water solutions to these circumstances, the authors found that the presence of ions reduces the efficiency of converting microwave energy into heat by suppressing dielectric polarisation (Anwar et al., 2015).

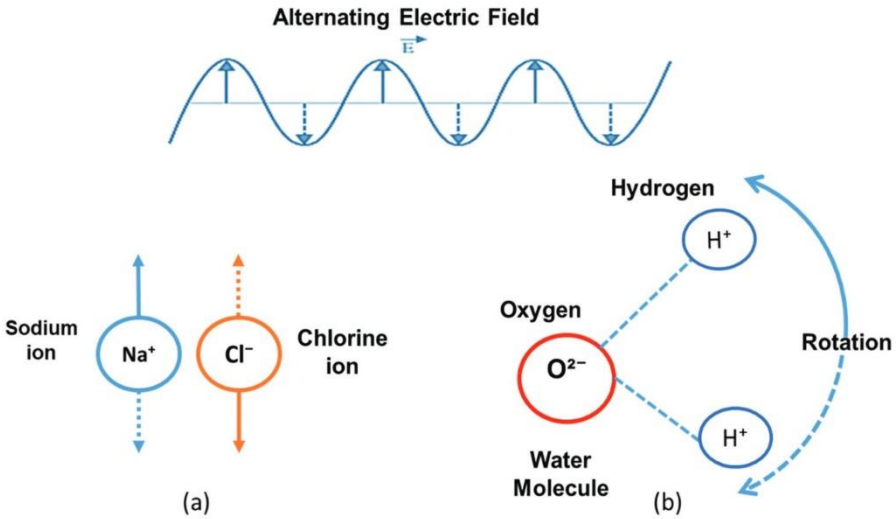

Figure 1.9. Heat generation by a) ionic conduction and b) dipole rotation under the action of an electromagnetic field; adapted from (Bou-Orm et al., 2021).

The position of the food in the microwave cavity is also important (Jiao et al., 2025; Pitchai et al., 2012; Taghian Dinani et al., 2021). To investigate how sample placement and orientation impact microwave

heating, Dinani et al. (2021) used gellan gel cubes containing Maillard reactants. They discovered that off-centre placements and vertical orientation, particularly in magnetron ovens, resulted in more even heating and greater energy absorption. A circular food container with 30 thermocouples embedded allowed Pitchai et al. (2012) to measure temperature rise at different locations inside microwave ovens in order to investigate heating patterns. Compared to samples at the centre of the turntable, they discovered that samples near the edge heated more quickly and evenly. Heating non-uniformity is primarily caused by hot and cold spots that form as a result of variations in the electromagnetic field distribution and material heterogeneity in multimode cavities (Jiao et al., 2025).

1.2.3.3.3. Dielectric properties of foods

The electrical properties of materials in the context of microwave and radiofrequency heating are known as dielectric properties, which give an indication of how they interact with electromagnetic energy when heated by microwaves and radiofrequency (Ahmed & Ramaswamy, 2007). Dielectric properties are crucial for food applications that involve new microwave or radio frequency heating techniques. The heating performance of foods is influenced by numerous factors when they are heated using microwaves or electromagnetic radiation. The dielectric properties evaluate the absorption of microwave energy by the material, and the heating behavior of foods. Food can be microwave-heated in microwave ovens, which is a useful, quick, and safe method that has been used extensively. The primary issue with this process is that various food components react differently to electromagnetic energy, and sometimes result in non-uniform heating of the food, which highly depends on the cavity, during microwave heating (Tıraş et al., 2019).

The dielectric properties of the food and the depth to which the microwaves penetrate the food material have a significant impact on how and to what extent food heats when exposed to microwave energy. Therefore, knowledge of how microwave energy interacts with food depends on dielectric properties (Ahmed & Ramaswamy, 2007). The dielectric constant (ε_r') and the dielectric loss factor ($\varepsilon_r^{"}$), which are the real and imaginary components of the relative complex permittivity (ε_r), respectively, are the dielectric properties. These are determined by the following equation:

$$\varepsilon = \varepsilon_0 \cdot \varepsilon_r = \varepsilon_0 \cdot (\varepsilon_r' - i\varepsilon_r^{"}) \qquad \textit{Eq. 1.4}$$

The dielectric constant and loss factor are often normalised relative to the dielectric characteristics of a void, ε_0, which allows relative dielectric properties (dielectric constants and loss factor) to be defined as dimensionless quantities (Curet, 2019).

$$\varepsilon''_{r(\sigma)} = \frac{\sigma}{2\pi \cdot f \cdot \varepsilon_0} \qquad \textit{Eq. 1.5}$$

$$\varepsilon''_r = \varepsilon''_{r(p)} + \varepsilon''_{r(\sigma)} = \varepsilon''_{r(p)} + \frac{\sigma}{2\pi \cdot f \cdot \varepsilon_0} \qquad \textit{Eq. 1.6}$$

where $\varepsilon''_{r(p)}$ reflects the loss factor related to the polarisation mechanisms of water molecules (dipolar rotation). $\varepsilon''_{r(\sigma)}$ reflects the loss factor related to the ionic conduction mechanism. It depends on the electrical conductivity (σ) measurable in the system. The electrical conductivity (σ) is related to the relative dielectric loss factor and characterises the conductive properties of free electrons (Curet, 2019).

These characteristics govern a food material's behavior when subjected to a microwave field; they show the material's capacity to reflect, transmit, or absorb microwave energy. A dimensionless number, ε'_r, measures how well food can store electromagnetic energy, while another dimensionless number, ε''_r, measures how well food can convert electrical energy into heat.

The loss tangent or dissipation factor ($\tan\delta$) is a second term frequently used to describe the heating behavior of materials under microwaves. In reality, when polarised materials orient toward the direction of the electric field due to intermolecular friction, the dissipation factor represents a phase-shifting condition of the materials. This demonstrates how electromagnetic waves can get into food, along with the extent to which heat is produced as a result (Bou-Orm et al., 2021). The dissipation factor is given by the following equation:

$$\tan\delta = \frac{\varepsilon''_r}{\varepsilon'_r} \qquad \textit{Eq. 1.7}$$

The volumetric power density absorbed by a food material can be expressed as:

$$P = \frac{1}{2} \cdot \omega \cdot \varepsilon_0 \cdot \varepsilon''_r \cdot E^2 \qquad \textit{Eq. 1.8}$$

where P is the microwave power density absorbed in W/ m^3 and E is the local electric field strength in V/m (Olaniyi, 2016).

The penetration depth (dp) is given by the equation below:

$$d_p = \frac{1}{2\pi \cdot f}\left[2 \cdot \mu_0 \cdot \mu_r \cdot \varepsilon_0 \cdot \varepsilon'_r \left(\sqrt{1 + \left(\frac{\varepsilon''_r}{\varepsilon'_r}\right)^2} - 1\right)\right]^{-\frac{1}{2}} \qquad \textit{Eq. 1.9}$$

where f is the frequency of the electromagnetic wave. The value of microwave penetration depth in relation to product thickness can be a useful parameter when designing a food product to optimise its microwave heating and also when evaluating heating uniformity (Curet, 2019).

Beyond macroscopic quality attributes, microwave heating may also induce structural modifications at the molecular level, particularly in starch components.

1.2.3.3.4. Effect of microwave on starch structure

Zhong et al. (2019) treated high-amylose maize starch with short-time microwave treatment (1-4 min), at 2450 MHz with the power of 1.2 kW, and observed structural and digestion-related changes on multiple scales. Short amylopectin chains and intermediate amylose chains were both partially broken down after just 1 min of treatment. The treated starch also displayed higher relative crystallinity under confocal laser scanning microscopy. Moreover, RS content increased and viscosity dropped in comparison to the native sample (i.e., lower digestibility). However, no additional molecular chain breakdown was seen when the treatment duration was extended to 2-4 min. During this phase, viscosity increased and RS content decreased (i.e, digestibility increased). Crystallinity significantly decreased, indicating disruption or realignment of crystalline regions. The authors attribute these alterations to internal heating caused by microwaves, rotations of water molecules, and structural rearrangements within starch granules. At first, the microwave energy causes crystalline lamellae to reorganise, increasing crystallinity; however, as exposure increases, the ordered regions start to collapse or realign, decreasing crystallinity and increasing enzyme access. These findings demonstrate how controlled irradiation can be used in microwave treatment to modify the structural and digestive characteristics of starch.

1.3. Quality of biscuits

Having described the composition and manufacturing process of biscuits, the following section focuses on quality parameters used to characterise the final product.

1.3.1. Physical parameters

1.3.1.1. Appearance

The significance of food appearance on food acceptance has been emphasised by Hutchings (Hutchings, 1977). A food's appearance is the first thing people notice before eating it. The food's palatability is determined by its appearance, so it matters. By merely observing a product, one can also evaluate certain aspects like texture or freshness. For this reason, producers and restaurateurs give their products and dishes aesthetic appeal a lot of thought (Hutchings, 1977). Similar to other food items, the appearance and eating qualities of biscuits determine the product's consumer appeal. Consumers do not like biscuits that are broken or that have been overbaked or underbaked, for instance (D. J. R. Manley, 1998b).

1.3.1.2. Water content

The main feature that sets biscuits apart from other baked products is their low water content (< 5% w.b.). The biscuit dough is made by adding water to flour and other ingredients, but it isn’t one of the main ingredients in the finished product. At high temperatures during baking, unbound water evaporates rather quickly. Such high temperatures may also partially release water that is bound to certain functional groups of the molecules of substances found in the bakery products, which aids in the final product's surface colouring. The stability of biscuits during storage, specifically their resistance to microbes and rheological characteristics, is always influenced by water activity and water content. Not only do biscuits have a low water content, but their water activity is also relatively low. Since biscuits have a long shelf-life, they are typically best kept at room temperature, though this can vary greatly depending on the climate circumstances (Cervenka et al., 2006).

1.3.1.3. Dimension

For the manufacturer, control over the biscuit's weight, biscuit dimensions, such as thickness and diameter, is indispensable. Significant fluctuations in biscuit thickness and weight can lead to excessive underweight and overweight packets that are against packaging regulations, as well as production breakdowns at the highly tolerated packaging stations (Cronin & Preis, 2000).

Variations in formula ingredients like sugar, fat, water, and protein have a significant effect on biscuit dimensions during baking, affecting weight, texture, and volume. For instance, higher protein content can decrease spread and increase thickness, while higher sugar and fat tend to thin and flatten biscuits by decreasing the dough viscosity and limiting the formation of the gluten network during baking, delaying dough setting and producing flatter, wider biscuits with a more friable structure. These dimensional shifts have an immediate impact on texture attributes such as fracturability and hardness, which are strongly correlated with sensory perceptions like bite force and crunchiness (Molina et al., 2021).

1.3.1.4. Specific volume

One of the most important physical characteristics of biscuits that indicates their overall structural quality, texture, and aeration is specific volume. It is commonly used as an indicator of baking performance and is defined as the volume-to-weight ratio of a dry matter. To measure the specific volume, the volume of the product must first be measured.

The density of a biscuit is generally reflected in its specific volume. Biscuits with a higher specific volume or a lower density are typically crispier and have a more appealing mouthfeel. Numerous elements, such as moisture content, starch gelatinisation, retrogradation behavior, and dough rheology, affect the specific volume of biscuits. While soft-textured doughs may expand rapidly during baking, but can collapse upon cooling, leading to a denser structure. Additionally, lowering the moisture content after baking can improve crispness by raising the biscuit's volume and specific volume (Hosney et al., 2023).

1.3.1.5. Texture

One of the main factors influencing biscuit consumers is texture. A crunchy feeling is produced when cracks form during biting due to the crumb structure, which frequently has a high percentage of air cells. Porosity or the percentage of air volume, can be used to characterise the crumb structure. Various instrumental techniques, including compression, bending, and puncture tests are used to capture the multi-parameter nature of biscuits in order to measure textural properties. One technique that is frequently used in the literature to assess biscuit texture is the three-point bending test (Salles et al., 2010).

1.3.1.6. Porosity

Heat and mass transfer, as well as the determination of mechanical properties, depend heavily on porosity, defined as the ratio of void volume to total volume in a material. Porosity has an impact on a

number of physical properties, including density, thermal diffusivity, thermal conductivity, and mass diffusion. Additionally, it affects the final dimensions and shape of products and contributes to the structural strength of dried foods. Characteristics such as crispiness and structure are also greatly impacted by porosity (Nemati & Takhar, 2025).

1.3.1.7. Colour

The sensory and quality perception of baked goods and biscuits is greatly influenced by colour. As a visual indicator of an ideal baking surface, browning is frequently the first feature that consumers consider. In addition to producing a desirable appearance, browning—which forms through Maillard reactions and caramelisation—also produces important flavour compounds, making a golden-brown crust both aesthetically pleasing and usefully informative about internal quality (Lukinac et al., 2022).

Modelling studies on cookies have demonstrated that surface colour changes, such as increased browning and decreased lightness, are closely related to heat transfer and moisture evaporation during baking, making them accurate indicators of the ideal baking point (Chen et al., 2024). To guarantee product consistency and customer satisfaction, manufacturers frequently monitor and regulate baking using objective colorimetric measurements (CIE Lab values and browning indices).

1.3.2. Biochemical parameters

1.3.2.1. pH

pH plays a vital role in controlling the AA formation in the food product, as the Maillard reaction is mainly influenced by pH. Lowering the pH by adding acids like citric acid can enormously reduce AA formation(Govindaraju et al., 2024). Additionally, factors such as pH and the presence of additional reactants can affect shelf life. As acidic environments are less conducive to microbial growth, for instance, lower pH values may enhance the antimicrobial effect (El Hosry et al., 2025).

1.3.2.2. Starch content

One of the primary ingredients in biscuits is starch. The rate at which starch is absorbed and the body's reaction to blood sugar are the primary indicators of the relationship between starch digestibility and human health. The GI classifies the amount of carbohydrates in various food types according to the blood glucose level after a meal. Long-term consumption of low-GI foods has been linked to lower rates of diabetes, heart disease, and some types of cancer, according to reports. Chronic disease prevention can be achieved by lowering the GI of food through specific methods. Previous research has identified a number of variables that can change a food's GI, such as food structure, the ratio of

amylose/amylopectin, the degree of food processing, and the presence of nutrients like fat, protein, and dietary fibre (Jia et al., 2020).

The amount of starch and the ratio of amylose/amylopectin have a significant impact on the quality of biscuits. It is also reported that incorporating exogenous starch into the reconstituted flour increased the dough's strength. Cookies with a high percentage of short amylose chains are thicker and have a lower spread ratio (Yang et al., 2022).

1.3.3. Nutrition parameters

1.3.3.1. Dietary fibres and their impact on dough

Dietary fibre is a class of compounds that consists of polymers of plant carbohydrates, including both polysaccharides and oligosaccharides. It includes components such as cellulose, hemicelluloses, pectic substances, gums, resistant starch (RS), and inulin. In addition, dietary fibre may be associated with lignin, which is a non-carbohydrate phenolic polymer. Carbohydrates are organic compounds composed of carbon, hydrogen, and oxygen, in which hydrogen and oxygen occur in approximately the same ratio as in water (H:O ≈ 2:1). Carbohydrates that are resistant to digestion in the small intestine are not hydrolysed or absorbed. In addition to resisting hydrolysis, digestion, and absorption in the human small intestine, dietary fibre enhances the bulk of the feces, promotes colonic fermentation, lowers postprandial blood glucose and insulin responses, and reduces preprandial cholesterol levels (Elleuch et al., 2011).

From improving digestive function to lowering the risk of chronic diseases, dietary fibre is essential for human health. Consuming enough fibre promotes bowel regularity, aids in blood glucose and cholesterol regulation, and benefits cardiovascular health. Additionally, short-chain fatty acids, which have advantageous metabolic effects, are produced when specific fibres are fermented in the colon. Therefore, fibre serves as a functional ingredient in the prevention of non-communicable diseases in addition to being an essential dietary component for preserving gut health. A workable strategy for enhancing public health outcomes is to increase fibre intake through frequently consumed foods such as baked goods (Lunn, Buttriss, 2007).

Dietary fibres affect bile acid absorption, transit time and nutrient uptake through their swelling and viscosity in the stomach and small intestine. Commensal bacteria produce the short-chain fatty acids butyrate, propionate, and acetate by fermenting fibre. Acetate and propionate enter the bloodstream and perform a number of tasks, such as providing energy and possibly promoting fullness in the brain's satiety centre. However, butyrate supplies energy to colon epithelial cells and influences cell signalling and immunological tolerance (Carlsen & Pajari, 2023).

In accordance with Regulation (EC) No 1924/2006 and the scientific assessment by EFSA, a cause-and-effect relationship has been established between the intake of dietary fibre and the maintenance of normal blood cholesterol concentrations. Maintenance of normal blood cholesterol concentrations is recognised as contributing to normal cardiovascular function in the general population (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2009).

Due to rising consumer awareness of nutrition and health, dietary fibre incorporation in baked goods has drawn more attention recently. Bread, cakes, and biscuits, in particular, which are frequently consumed and can be excellent carriers for fibre enrichment, are products that have fibre added to improve their nutritional profile. The main objective is to boost fibre consumption without materially sacrificing these products' organoleptic (taste, texture, and appearance) attributes (Sudha et al., 2007). The mechanisms of dietary fibres in the gastrointestinal tract are depicted below (Figure 1.10).

The use of cereal-based fibres, including oat, wheat, and rice bran, in biscuit formulations was investigated in a study conducted in 2007 (Sudha et al., 2007). The results showed that careful ingredient level optimisation could maintain acceptable product quality even though fibre inclusion changes the properties of the dough, lowers the spread ratio, and increases hardness. Similarly, a study investigated the presence of psyllium fibre in biscuits and discovered that up to a certain point, consumers continued to find fibre-enriched biscuits to be very palatable, suggesting that this is a promising path for the development of functional foods (Fradinho et al., 2015).

Additionally, as fibres are partially indigestible, their addition provides technological advantages like water retention, caloric dilution, and shelf-life extension (Elleuch et al., 2011). However, excessive incorporation may result in unfavourable changes to the texture or flavour of the product, making the use of emulsifiers or hydrocolloids necessary to counteract such effects. To make baked goods high in fibre, both nutrient-dense and aesthetically pleasing, more research is being done on striking a balance between health advantages and sensory qualities.

1.3.3.2. Glycaemic index (GI) and impact of baking on it

The idea of the glycaemic index (GI) as a system for ranking carbohydrates according to their direct effect on blood glucose levels was introduced in the early 1980s (Jenkins et al., 1981). GI was first created as a food selection guide for diabetics, informing them to choose foods with a low GI (Venn & Green, 2007). The impact of the total quantity of carbohydrates consumed is now also considered by the GI concept. Carbohydrate quality and quantity are reflected in the glycaemic load. It is computed by multiplying the GI by the total amount of carbohydrates that are available in a given quantity of food (Gerontiti et al., 2024). It indicates how much glucose is available for storage or energy after a

meal high in carbohydrates (Venn & Green, 2007). Low GI and glycaemic diets have been frequently suggested in recent years for the treatment of cardiovascular risk factors, particularly dyslipidaemia, as well as for the prevention of chronic diseases like diabetes, obesity, cancer, and heart disease (Jenkins et al., 2002).

A food's ability to raise blood sugar levels is measured by its GI, which is compared to the same amount of a reference food (white bread or pure glucose) (Priyadarshini et al., 2022). Foods are categorised as low GI (GI ≤ 55), medium GI (56 ≤ GI ≤69), or high GI (GI ≥ 70) (Priyadarshini et al., 2022; Venn & Green, 2007).

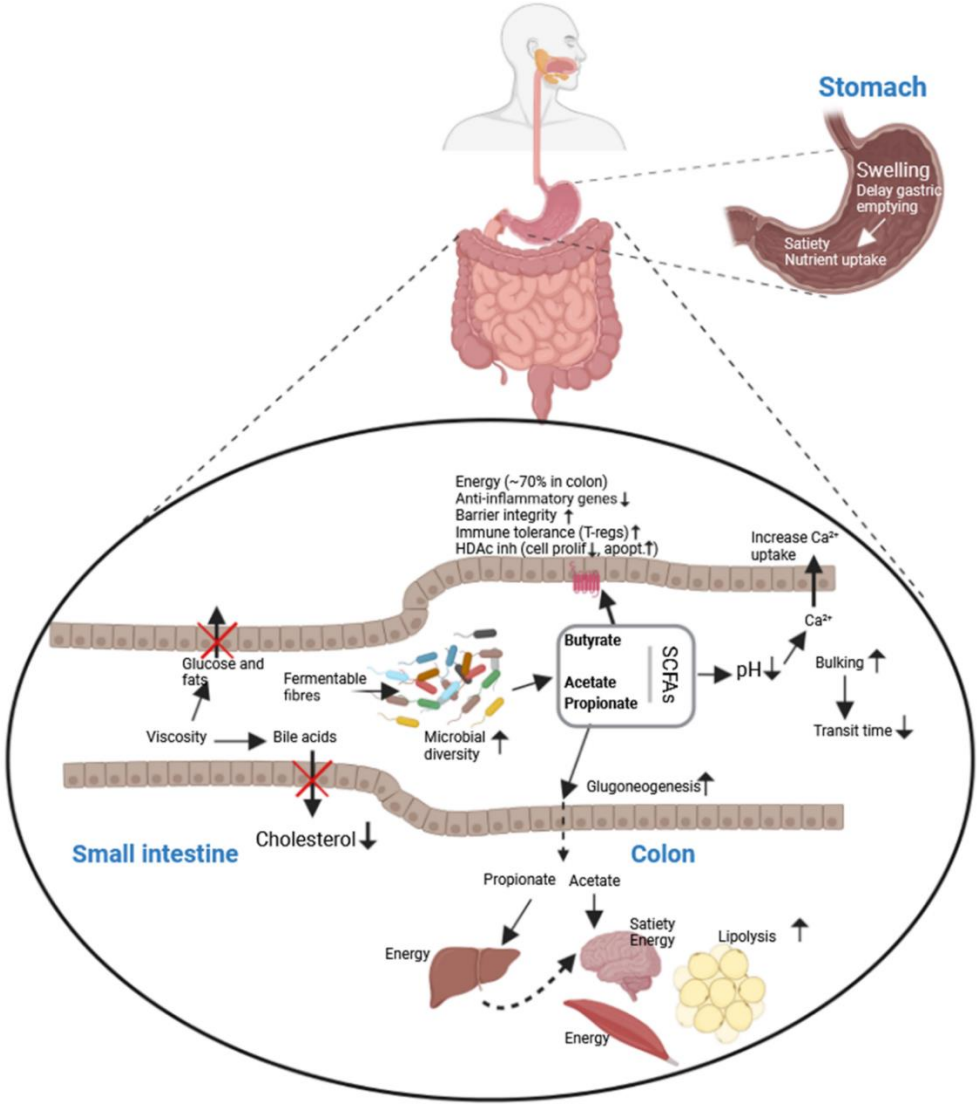

Figure 1.10. Mechanisms of dietary fibres in the gastrointestinal tract; In the stomach and small intestine, swelling and viscosity of fibres affect transit time, nutrient uptake, and bile acid reuptake. Colonic short chain fatty acids (acetate, propionate, and butyrate) are made through fibre-fermentation by commensal bacteria. Butyrate provides energy for colon epithelial cells and affects cell signaling and immune tolerance, whereas acetate and propionate are taken up into the blood to exert various functions including energy provisions and possibly satiety in the brain satiety center(Carlsen & Pajari, 2023).

The mouth is where digestion begins, and the stomach is where food is further broken down into smaller pieces (Figure 1.11). After being absorbed in the intestine, monosaccharides enter the bloodstream. The blood carries the majority of glucose, and the muscles and liver store the excess as glycogen. The composition and structure of foods with a high or low GI affect digestion and metabolism, which is reflected in glycaemic levels in response. The oral, stomach and intestinal phases are the three crucial phases of food digestion (Priyadarshini et al., 2022).

Biscuits are a widely consumed snack enjoyed by people all over the world. They are typically made with a high amount of fats and sugar to enhance their flavour, making them a popular choice among consumers. However, it is important to note that regular consumption of biscuits can increase the risk of obesity, which in turn can lead to the development of type 2 diabetes, high cholesterol, and coronary heart disease (Vuji et al., 2014). In light of this, the food industry has been paying attention to current trends and the recommendations of nutritionists, who emphasise the preventive and protective role of a healthy diet in combating various diseases. As a result, food technology specialists have been focusing their efforts on developing biscuits with functional properties that offer additional health benefits.

Starch, a major plant metabolite, is the primary carbohydrate in the human diet. It can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS for nutritional purposes. RS, which is not broken down in the small intestine, is instead fermented by bacterial microflora in the large intestine. This fermentation impacts various physiological functions and offers several health benefits, such as lowering glycemic and insulinemic responses, hypocholesterolemic effects, and protection against colorectal cancer (Sánchez-Pardo et al., 2007). A diet rich in slow-digestible carbohydrates could potentially help prevent chronic diseases such as obesity.

Some studies have explored techniques for reducing the GI of cereal products (Sánchez-Pardo et al., 2007). For instance, a study on pound cake compared the impact of cooking methods (microwave vs. conventional oven) on starch bioavailability in fresh and stored pound cake crumb. It was revealed that microwave-baked pound cake had a 24% lower predicted GI compared to traditionally-baked pound cake (59.13 vs. 77.92%) (Sánchez-Pardo et al., 2007). Vuji et al. (2014) investigated the potential for creating lower GI cookies by substituting 30% of white wheat flour with pseudo-cereals (buckwheat or amaranth flour) and legumes (soy or locust bean flour). The findings indicated an improvement in macronutrient quality and a significant increase in RS content with the use of pseudo-cereals (43.5-56.1%).

The impact of shortcrust biscuits enriched with apple pomace on the GI was examined by Alongi et al. (2019). The outcomes demonstrated a significant reduction in the expected GI of the reformulated

biscuits due to the inclusion of apple pomace. Initially, the traditional cookie had a GI of 70, placing it in the high GI category. However, by substituting wheat flour with apple pomace at 10% and 20%, the GI of the biscuits decreased to 65 and 60, respectively, resulting in a GI reduction of approximately 14%.

Olawoye et al. (2020) showed that there is a correlation between biscuit texture, RS, and GI. In their study, the effect of baking temperature and time on the RS, GI, and glycaemic load of gluten-free biscuits was evaluated. They observed that the increase in RS and decrease in predicted GI of cookies were associated with low temperature and high baking time. The results of this research revealed that the breaking strength of biscuits increased with increasing baking time rather than baking temperature. The increase in the RS fraction of biscuits could be attributed to the strong interaction between molecular chains and the helical structure, causing orientation and starch crystal formation after high-temperature baking. The formation of starch crystals in biscuits would lead to a resistance of biscuits to enzymatic hydrolysis, resulting in an increase in RS (Olawoye et al., 2020).

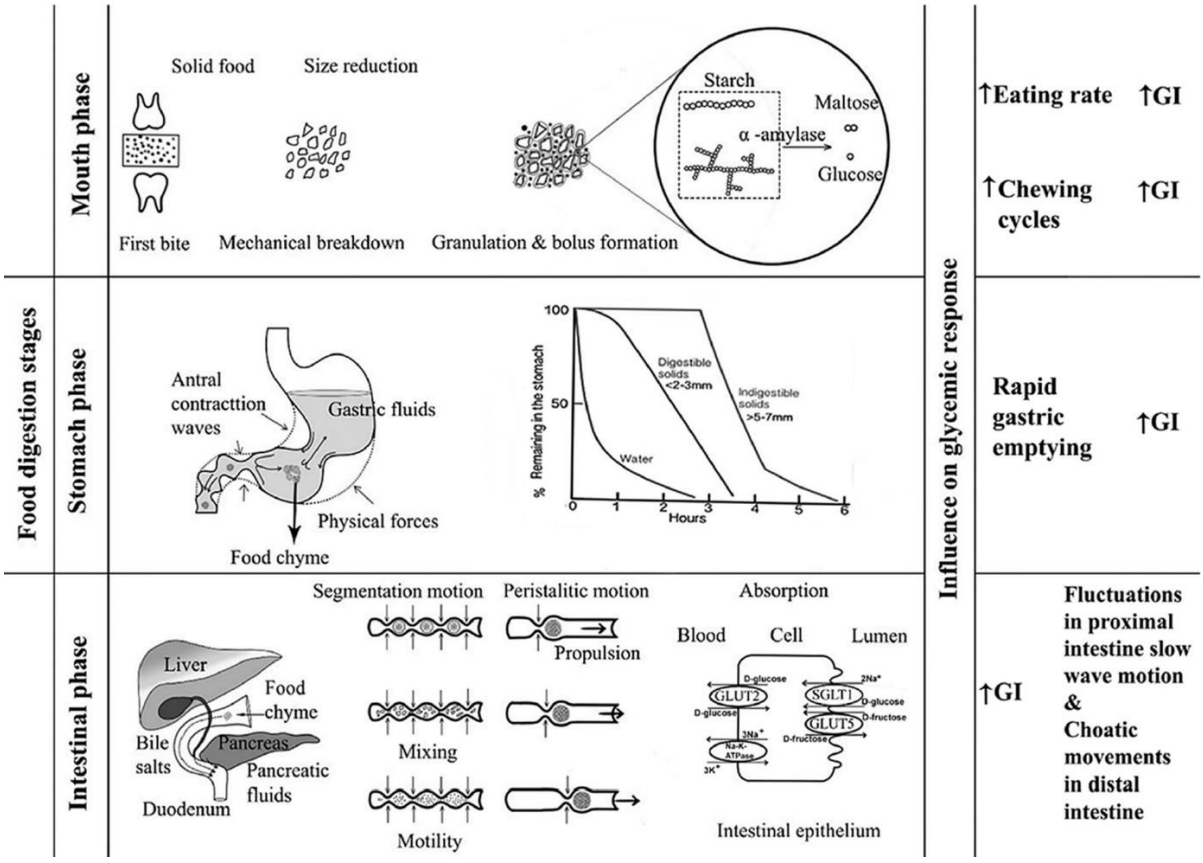

Figure 1.11. Digestion stages and their influence on GI; food digestion involves three important stages: oral phase, stomach phase, and intestine phase (Priyadarshini et al., 2022).

Beyond technological quality, thermal processing may induce the formation of neo-formed contaminants (NFCs), which are discussed in the following section due to their relevance for both product safety and formulation strategies.

1.3.4. Neo-formed contaminants (NFCs)

NFCs are common in many foods, especially those subjected to high-temperature processing. Among these contaminants, products arising from the Maillard reaction, sugar reduction, thermal degradation of polyphenols, and lipid oxidation, including AA, furan, furfuryl alcohol, and hydroxymethylfurfural, are consistently linked to potential neoplastic effects. NFCs are found in globally traded commodities like coffee and cocoa, posing a significant risk due to their frequent consumption by consumers. A direct correlation exists between consumption frequency, exposure levels, and health risks. Hence, it's crucial to establish reliable methods to determine levels in both matrices, aiming to mitigate their formation and minimise risks to consumers (Medina-Orjuela et al., 2024).

Health and risk control bodies have developed guidelines to minimise NFCs exposure. Since 2017, EFSA has set benchmark food AA levels. Specifically, 400 µg/kg for roast coffee and 850 µg/kg for instant soluble coffee. Similarly, since the mid-80s, the California Office of Environmental Health Hazard Assessment (OEHHA) has mandated warning labels for toxic substances such as AA in food (Medina-Orjuela et al., 2024).

1.4. Innovation in biscuit manufacturing

Based on the previously described product, process, and safety considerations, innovation strategies in biscuit manufacturing are presented, particularly those related to formulation and mitigation of undesirable compounds.

1.4.1. Formulation

The replacement of wheat flour by dietary fibre from various sources can change physicochemical, textural, and organoleptic characteristics of bakery products (Drakos et al., 2021; Mesias et al., 2024; Molavi et al., 2015; Papageorgiou et al., 2020; Pasqualone et al., 2019; Szabłowska & Tanska, 2016).

Molavi et al. (2015) examined the effects of substituting up to 10% of treated acorn flour with wheat flour in sponge cake recipes. Although adding acorn flour improved the cakes' functional qualities, it also made them harder, chewier, and denser while decreasing their cohesiveness and springiness. The cake's sensory qualities were adversely affected by these textural alterations, especially at higher substitution levels. The study came to the conclusion that in order to maintain acceptable texture and palatability, acorn flour should be kept to below 10% in cake formulations. This contrast demonstrates that although acorn flour is a useful functional ingredient, its use needs to be customised for the particular kind of baked good.

Apple pomace is the main by-product produced in the apple fruit processing industry. It is a rich source of carbohydrate, pectin, crude fibre, and minerals. The dietary fibre content in apple pomace ranges between 33 – 35% (Szabłowska & Tanska, 2016). The influence of hydrated commercial dietary fibre on wheat dough rheology (5, 10, and 15% flour replacement) and physical and sensory properties of cookies was examined by Szabłowska and Tanska (2016). It was found that the addition of hydrated apple powder significantly increased the rheological properties of dough, such as water absorption (from 58.0% to 75.3%) and dough stability (from 6.7 min to 11.6 min). It was also concluded that hydrated apple powder addition reduced physical properties of cookies, such as volume (from 10.4 cm^3 to 8.0 cm^3), diameter (from 4.7 cm to 4.2 cm), volume index (from 1.35 cm to 1.10 cm), and porosity (from 0.32 to 0.24). Sensory properties (taste, odour, stickiness, firmness, and density) of cookies were also analysed. Cookies with the addition of hydrated apple powder had a fruity taste and odour and showed high overall acceptance. From this study, it was found that hydrated apple powder can be used as a potential source of dietary fibre in cookie formulation (Skaltsi et al., 2022).

The incorporation of acorn flour in biscuits at substitution levels between 30% and 60% was examined by Pasqualone et al. (2019). According to their findings, acorn-enriched biscuits exhibited higher levels

of antioxidant activity and phenolic compounds, which enhanced their oxidative stability. In terms of texture, these biscuits were noticeably more friable, more voluminous, and darker in colour—qualities that are frequently preferred in biscuits. While the bitterness and astringency stayed low, the addition of acorn flour also improved the bran-like and caramel scents, most likely as a result of Maillard reactions. The biscuits retained good sensory acceptance overall, indicating that acorn flour can be used in biscuit formulations to enhance textural appeal and nutritional value at relatively high levels.

Papageorgiou et al. (2020) investigated up to 70% roasted carob flour incorporation in cakes as a substitute for cocoa powder and as a partial substitute for wheat flour. The final cakes had textural and sensory characteristics similar to the cocoa-containing control at substitution levels of 30–50%. In particular, the cakes' volume, softness, and mouthfeel were all acceptable, and the roasted carob flours shared volatile compounds—furans and aldehydes—contributed pleasing cocoa-like scents. These results demonstrate that carob flour can serve as a flavour enhancer, partial fat substitute, and nutritional booster, particularly in chocolate-style baked goods.

In a study conducted by Drakos et al. (2021), they found that adding inulin and barley flour to biscuit dough increased the weight and softness of the biscuits, but the spread ratio and product colour changed. They explained that combining different fibre sources increased the overall phenolic content, showing that these fibres can improve functional and nutritional qualities as long as substitution levels are optimised to preserve product quality.

More recently, Mesías et al. (2024) assessed the production of wheat-based biscuits enriched with coconut sugar, chia seeds, and 10% carob flour. This combination resulted in a fourfold increase in dietary fibre and a notable improvement in antioxidant capacity. Beyond just providing basic nutrition, the carob-enriched biscuits also showed antifungal qualities. The sensory evaluation verified that carob flour maintained overall acceptability, indicating that it did not negatively affect texture, flavour, or appearance when used at moderate levels despite the study's primary focus on biochemical and bioactive characteristics.

Overall, previous studies show that the incorporation of fibre-rich ingredients, such as acorn and carob flours, can enhance the nutritional and functional quality of bakery products while maintaining desirable sensory properties when used at appropriate levels. The findings of reviewed studies show that acorn flour offers high concentrations of antioxidants, phenolic compounds, and dietary fibre, all of which improve oxidative stability and nutritional value. Conversely, the natural sweetness, cocoa-like flavour, and extra fibre and minerals found in carob flour can enhance the product's antioxidant capacity while lowering the need for added sugar and fat. By incorporating these two flours, a biscuit with better health-promoting properties can be produced.

1.4.2. Mitigation of acrylamide (AA)

1.4.2.1. Ammonium bicarbonate (ABC) substitution with sodium bicarbonate (SBC)

AA in biscuits represents a major concern. Amrein et al. (Amrein et al., 2004) considered the effects of ingredients, additives, and processing parameters on the formation of AA in gingerbread. In this research study, the source of reducing sugars and free asparagine was determined, and it was assessed how various LAs affected the production of AA. They observed that ABC significantly accelerated the formation of AA, and both the amount of AA and the degree of browning increased with baking time and were correlated. By using SBC as an LA, the concentration of AA was lowered by more than 60%. AA formation was limited by free asparagine; however, the amount of AA could be reduced by adding organic acids or substituting sucrose with reducing sugars. Using SBC as an LA, reducing free asparagine, and avoiding prolonged baking were found to significantly reduce AA in gingerbread.

Furthermore, a modification of the current formulation of biscuits to reduce the AA content while maintaining the chemical, physical, and sensory characteristics of the original product was investigated by Lo Faro et al. (2022). The approach used in this study was modelled after the FoodDrinkEurope Acrylamide Toolbox. Three factors were assessed using a factorial design of experiment: the amount of ABC, the baking temperature program, and the time duration that steam was released during the baking process. A 87.2% decrease in AA concentration was achieved by lowering the temperature during the central baking phase (from 170°C to 150°C), releasing steam for 3 min, and partially substituting SBC (from 4.5 g to 12.48 g) with ABC (from 9.0 g to 1.5 g per 500 g of flour). The parameters that demonstrated the strongest correlation with the amount of AA in biscuits were CIELab colour indices and a_w. They suggested these parameters as markers to predict the amount of AA along production lines for a real-time assessment.

1.4.2.2. Replacing leavening agents (LAs) with CO_2 mixing

The use of CO_2 gas hydrates as an LA instead of ABC was investigated in a research study by Kollemparembil et al. (2023) to make black-and-white cookies. To find the ideal amount for a good leavening effect, three different gas hydrate concentrations—20%, 40%, and 50%—were used. The sudden drop in temperature caused by the gas hydrates addition, however, had an unacceptably negative impact on the cookie dough. In order to introduce CO_2 into the mixing unit, this gas was injected through the bottom valve of the reactor. In order to run the system as a bubble column, the gas cylinder was opened until the reactor's ideal pressure (36–37 bars) was reached. The reactor lid's upper vent valve was also left open to allow for continuous gas bubbling in the water. The synthesis of gas hydrates began with nucleation and lasted for three hours after the gas molecules were dissolved at a low temperature (0–1°C). It was reported that the use of 50% gas hydrates as an LA resulted in

cookies with a specific volume more than half that of cookies made with ABC. Springiness and hardness, two textural properties of the pastry that determine quality, stayed within an acceptable range in the gas hydrate-containing cookies. Through this study, the amount of AA was decreased from 24.8 µg/Kg to approximately 18 µg/Kg. This study showed that CO_2 gas hydrates can be used as an LA in black-and-white cookies.

1.4.2.3. Process modification

The possibility of removing AA from foods by exploiting its chemical and physical properties was studied by Anese et al. (2010). Commercial potato chips and biscuits were vacuum-treated at various pressures, temperatures, and time combinations. The results demonstrated that only samples that had been previously hydrated at water activity values greater than 0.83 were able to remove AA, and that the maximum amount of AA removal was attained after 5 to 15 min of vacuum treatment at 6.67 Pa and 60°C. It was feasible to eliminate 43% and 18% of the AA from the biscuits and potato chips, respectively, by using these process parameters. A similar process was applied to cookies to reduce the AA content in them (Yıldız et al., 2017). In comparison to the conventionally baked sample, which had an AA concentration of 445 ng/g, cookies made using vacuum-combined baking had an AA concentration of approximately 300 ng/g, which is about 30% lower. Upon lowering the pressure, a minor drop in the convective heat transfer coefficient was noted. The limited temperature increase and consequently the mitigation of AA were found to be primarily caused by the evaporative cooling effect produced by a sudden pressure reduction.

The impact of fennel seeds and black cumin seeds addition into the formulation of conventional and microwave-baked biscuits on the nutritional, physical, and sensory properties was investigated in a research conducted by Al-Ansi et al. (2019). Total phenolic compounds, antioxidant activity, and AA contents of products were measured. Microwave biscuits were baked at 700 W for 90 s. The findings showed that in comparison to conventionally baked biscuits, microwave-baked biscuits had lower AA content and higher antioxidant activities. However, when black cumin seed was added, the amount of AA in the conventionally baked biscuits decreased from 355.2 µg/kg to 138.6 µg/kg, and in the microwave-baked biscuits, it decreased from 306.9 µg/kg to 97.8 µg/kg. Low AA content and high antioxidant activity demonstrated the benefits of microwave baking and adding fennel seeds or black cumin seeds to biscuit recipes.

Dong et al. (2022) studied the influence of microwave power and microwave time on the formation of thermal processing hazards (TPHs) and their precursors was explored in microwave-baked biscuits. According to the findings, the amount of AA, 5-hydroxymethylfurfural, methylglyoxal, and 3-deoxyglucosone increased linearly as microwave power (440, 480, and 520 W) and microwave time (2,

2.5, and 3 min) were increased. Among the four TPHs, there was a noteworthy correlation. It was observed that the other three TPHs can be formed either directly or indirectly by 3-Deoxyglucosone. A comparison was made between the amount of TPHs produced by microwave baking and traditional baking at the same texture level. Microwave baking at 440 W for 3 min effectively reduced the content of methylglyoxal, 3-Deoxyglucosone, AA, and 5-hydroxymethylfurfural by 60.75, 30.19, 30.87, and 61.28% respectively, when compared to traditional baking (190°C, 7 min).

The reviewed studies show that AA formation in bakery products can be significantly reduced while maintaining acceptable product quality through both process modifications, like microwave baking, and ingredient modifications, like partially substituting ABC with SBC or incorporating CO_2.

In the present thesis, two novel methods for reducing the formation of AA in biscuits are examined: microwave baking and over-pressure mixing with CO_2. By applying CO_2 over-pressure mixing to partially substitute ABC with CO_2, the goal is to reduce the amount of AA precursors linked to ammonium-based LAs while preserving sufficient dough expansion and texture. By lowering the overall baking temperature, microwave baking is investigated as a non-traditional baking method that can lower AA levels by minimising exposure to high thermal conditions that cause AA formation. Evaluating these two methods allows for a clearer understanding of their impacts on AA reduction in biscuits and possible use in producing safer and healthier baked goods.

1.5. State of the art's conclusion

Asparagine and reducing sugars undergo Maillard reactions during baking, which is the main source of AA, a thermal processing contaminant. Its release in baked goods raises serious concerns about food safety and motivates researchers to develop methods that reduce AA formation while preserving the sensory and physical quality of the final product. Early work by Gökmen et al. (Gökmen et al., 2007) demonstrated that dough formulation and baking conditions, such as reducing the concentration of reducing sugars and lowering baking temperature, significantly influence both AA and hydroxymethylfurfural formation in cookies, suggesting that process and recipe adjustments are crucial for mitigation. Furthermore, Gökmen et al. (Gökmen et al., 2008) found a strong correlation between browning ratio and AA levels in model cookies, indicating that colour development could serve as an indicator of AA formation under different baking conditions. More recent studies have focused on ingredient modifications and process adjustments to achieve this balance. For instance, Lo Faro et al. (2022) demonstrated that partially substituting ABC with SBC, lowering the baking temperature from 170°C to 150°C, and introducing steam for 3 min reduced AA by approximately 87%.

Alternative leavening strategies have also shown potential in reducing AA precursors. Kollemparembil et al. (2023) explored the use of CO_2 gas hydrates as a partial replacement for ABC in black-and-white cookies. Their study revealed that 50% substitution lowered AA while maintaining acceptable textural properties, although a decrease in specific volume was observed. This approach demonstrates that replacing ABC with novel dough aeration methods has the potential for producing healthier biscuits.

In addition to ingredients, process modifications can substantially mitigate AA formation. Vacuum-assisted baking has been reported to decrease AA levels due to evaporative cooling that limits thermal exposure (Anese et al., 2010; Yıldız et al., 2017). Microwave baking, combined with the addition of fennel or black cumin seeds, has been shown to lower AA and other thermal processing hazards (AL-Ansi et al., 2019; Dong et al., 2022).

In summary, these strategies—including partial ABC substitution, CO_2 mixing, and microwave baking—lead the present investigation into novel approaches to reduce AA formation in biscuits, offering the potential to produce safer, healthier baked products with lower energy consumption.

2. THE CONTEXT, POSITIONING, AND SCIENTIFIC APPROACH

2.1. Project context and positioning

The scientific literature on the baking of dry cereal-based products such as biscuits is considerably less extensive than for high-moisture products like bread and cakes. Conventional baking science has focused on mechanisms of heat and mass transfer, rheology, and quality formation in moist systems (Datta & Rakesh, 2013), whereas low-moisture, sugar-rich matrices behave differently due to limited water mobility and different energy absorption characteristics.

Microwave heating is an alternative technology that delivers energy volumetrically, enabling rapid internal temperature rise and reduced processing times (Datta & Rakesh, 2013). Microwave energy interacts with polar molecules and ions in the food matrix, producing dielectric heating that depends on the dielectric properties of the material (Jiang et al., 2018). Dielectric properties can vary strongly with moisture content, frequency, and temperature — making microwave interactions in low-moisture systems fundamentally different from those in bread or cakes (Torrealba-Meléndez et al., 2015).

Microwave-infrared combined baking has been explored for products such as cakes and breads, showing improvements in browning and texture (Demirekler et al., 2004; Sumnu et al., 2005). However, microwave baking of biscuits — especially low-moisture types — remains under-studied, particularly with regard to thermal hazards (e.g., AA) and energy efficiency.

AA formation is a significant concern in baked cereal products. AA arises primarily from Maillard reactions involving reducing sugars and asparagine during high-temperature processing (Amrein et al., 2004). Studies have shown that modifying baking conditions (e.g., temperature profile, steam) and formulation (e.g., LAs) can reduce AA levels in baked goods (Lo Faro et al., 2022). However, microwave baking as a mitigation strategy for AA in low-moisture biscuits has not been thoroughly evaluated.

In addition, alternative leavening strategies such as CO_2 over-pressure mixing have been proposed to reduce reliance on chemical leaveners like ABC, which is known to contribute to AA formation (Amrein et al., 2004). SBC can replace ABC but introduces sodium and often requires acidulants such as pyrophosphates for functionality. CO_2 over-pressure mixing may allow gas incorporation without these issues, potentially leading to new porous structures and cleaner label products, but its effectiveness in biscuits has yet to be fully characterised.

Furthermore, modifications in processing and formulation can influence the digestibility and the GI of starch-based products. Processing methods such as microwave heating have been shown to affect starch structure and digestibility, which in turn can impact nutritional responses (Sánchez-Pardo et al., 2007). These nutritional considerations add further impetus to exploring novel processes.

Taken together, these points situate the present study at the intersection of microwave processing, alternative leavening, product quality, safety (AA mitigation), and nutritional outcomes — a nexus that is largely unexplored for low-moisture biscuit matrices.

2.2. Scientific problem and research questions

Although process innovations have been proposed in baking science, key knowledge gaps remain specifically for low-moisture biscuits:

- CO_2 over-pressure mixing as an alternative to chemical leavening has not been systematically investigated for its impact on dough rheology, expansion, and final product quality.
- The interaction of microwave energy with low-moisture doughs and its effects on heat transfer, starch transformation, and browning reactions are not well understood.
- Quantitative comparisons of AA formation between microwave and conventional baking under controlled quality conditions are not well studied.
- The effects of processing innovations on starch digestibility fractions and eGI require further exploration.
- Energy consumption and efficiency comparisons between microwave and conventional baking have not been fully established.

Central Scientific Problem:

How can microwave baking and CO_2 over-pressure mixing be developed and optimised for biscuit production to reduce AA formation, control texture and structural attributes, improve nutritional properties (e.g., eGI), and enhance energy efficiency compared to conventional baking?

From this scientific problem, the following research questions are formulated:

1. Leavening Strategy: Can CO_2 over-pressure mixing reduce or eliminate the need for ammonium-based chemical leaveners while achieving adequate expansion and acceptable texture?
2. Dielectric Heat Transfer: How do the dielectric properties of low-moisture dough influence microwave heating behaviour and resultant heat distribution?
3. AA Mitigation: To what extent can microwave baking reduce AA formation compared to conventional baking under matched quality conditions?
4. Nutritional Impact: How do microwave and CO_2 mixing affect starch digestibility fractions and the eGI?
5. Energy Efficiency: What are the specific energy requirements of microwave baking relative to conventional methods?

2.3. Project objectives and scientific approach

This PhD project targets six primary objectives:

1. Produce biscuits with reduced or no chemical LAs via CO_2 over-pressure mixing and evaluate the feasibility and functionality of the process.
2. Evaluate the potential of microwave treatment for baking biscuit-type matrices and define optimal conditions related to structure, texture, and temperature distribution.
3. Assess the potential of the processes to reduce AA, a known thermal processing hazard, and eGI.
4. Evaluate the impact of incorporating fibre-containing flours into the formulation on product quality and functionality.
5. Assess the effects of processing and formulation changes on sensory quality, including appearance, aroma, taste, and texture.
6. Compare the energy consumption of microwave baking with conventional baking methods and identify strategies for energy efficiency.

The scientific approach is structured around these objectives and illustrated by the conceptual framework below.

The study investigates two complementary strategies:

- Formulation Modification:

The focus is on reducing chemical LAs, particularly ABC, which contributes to AA formation. CO_2 over-pressure mixing is applied specifically to laminated biscuits, which contain higher water and lower fat content compared to rotary biscuits. Since CO_2 is more soluble in fat than in water (Chaix et al., 2015), this compositional difference makes laminated biscuits more challenging for gas incorporation, thus requiring careful optimisation of CO_2 mixing to achieve adequate gas retention (~8 cm^3 per g of dough) and promote the formation of new porous structures. This strategy supports the objective of reducing chemical leaveners while maintaining desirable biscuit structure and sensory quality, contributing to "clean-label" reformulations.

- Process Modification:

Microwave baking is explored as an alternative baking method that enables faster heating and lower baking temperatures. These conditions can reduce the Maillard reaction intensity and subsequently limit AA formation. Combined microwave-infrared baking is also studied to optimise final product browning and texture while improving energy efficiency. This supports objectives related to defining

optimal baking conditions, reducing AA and eGI, and decreasing energy consumption during baking (Figure 2.1).

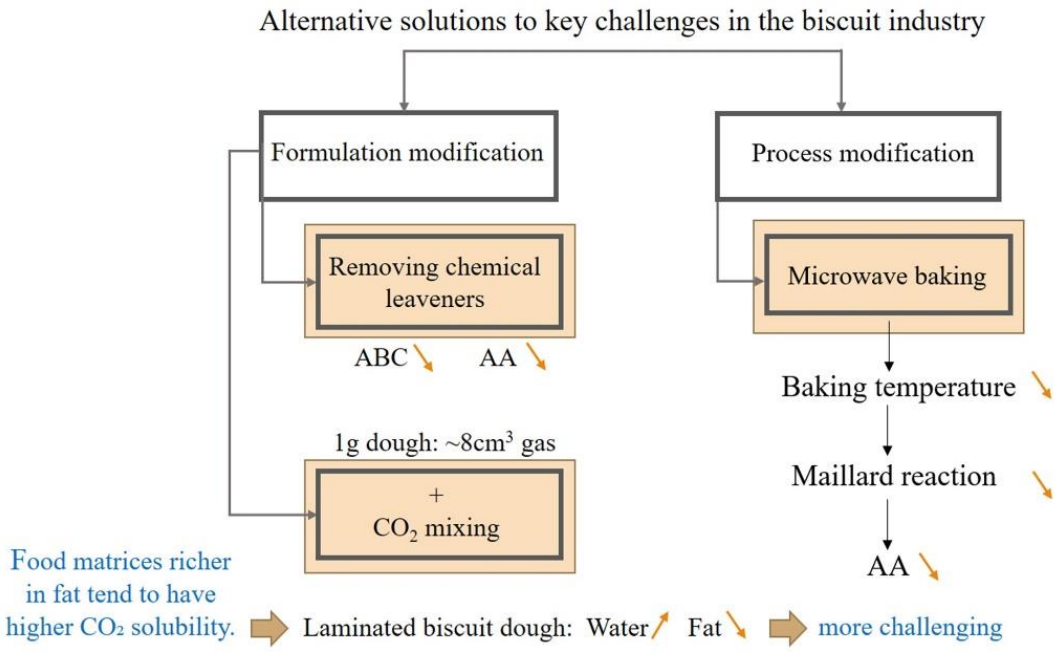

Figure 2.1. The scientific approach; (ABC: ammonium bicarbonate; AA: acrylamide).

This approach will be evaluated experimentally through controlled formulation trials, dough preparation under various conditions, and comprehensive product characterisation, including physico-chemical, sensory, and energy consumption analyses.

3. MATERIALS AND METHODS

3.1. Selection of flour, optimal mixing, and baking conditions

The first part of the Materials and Methods considers the primary ingredients for making biscuits, including the selection of suitable flour, and optimal dough mixing and baking conditions. Both laminated and rotary biscuit doughs containing LAs (LB-ABC and RB-ABC) are evaluated. The optimal mixing conditions for the LAs-free laminated biscuit dough (LF-LB-CO2) and the laminated biscuit dough with SBC (LB-CO2) under CO_2 over-pressure mixing are also discussed.

3.1.1. Primary ingredients for biscuit dough preparation

3.1.1.1. Flour

Three types of flour were tested to determine the most suitable for maintaining the biscuits' density below 0.3 g/cm³, in accordance with French biscuit industry standards (ARVALIS et al., 2022). It should be noted that lower density results in a more crumbly, airy product. The flours T65 (Evelia, France), T55 (Paulic, France), and T55 (Enilia Bio, France) were sourced from the suppliers. Some characteristics of flour T65 (Evelia, France), including the protein content and P/L, were provided by the supplier, while the remaining parameters were measured in the GEPEA laboratory using an Alveograph (Chopin Technologies, France). The characteristics of the flours Paulic (France) and Enilia Bio (France) were obtained by sending samples to an external laboratory.

3.1.1.2. Additional ingredients

In addition to flour, biscuit doughs were prepared using butter with 82% fat matter (Président, France), icing sugar and granulated or table sugar (Béghin Say, France), salt, water, glucose syrup (Vahiné, France), ABC (Brenntag, Germany), SBC (Brenntag, Germany), SAPP10 (Budenheim, Germany), and skim milk powder with 1.5% fat (MATINES, France). The SAPP10 has been selected as a slow-acting agent, releasing just 10% of the CO_2 from SBC during dough mixing.

3.1.2. Formulation

Several recipes were tested to adapt them to the forming machines and the quality of the final products. Five categories of biscuits were made:

Laminated biscuits containing LAs (LB-ABC): The dough was mixed at atmospheric pressure. The LB-ABC biscuit is the positive control.

Laminated biscuits free from LAs (LF-LB): The dough was mixed at atmospheric pressure. The LF-LB is the negative control.

Laminated biscuits free from LAs with CO_2 over-pressure mixing (LF-LB-CO2) with and without gas flow: The dough was mixed under pressure (0.5 bar) with 100% CO_2. During the mixing with gas flow, the mixer valve was kept partially open during mixing and kneading to allow continuous gas renewal. In contrast, for mixing without gas flow, the mixer valve was kept closed.

Laminated biscuits free from ABC containing SBC and SAPP 10 with CO_2 over-pressure mixing (LB-CO2) with and without gas flow: The dough was mixed under pressure (0.5 bar) with 100% CO_2.

Rotary biscuits containing LAs (RB-ABC): The dough was mixed at atmospheric pressure.

The composition of these biscuits is expressed per 100 g (w.b.) of flour (Table 3.1).

For the over-pressure mixing, CO_2 gas at a pressure of 0.5 bar was applied. 0.5 bar is the maximum pressure level allowed by the mixer.

Table 3.1. Biscuit ingredients per 100 g of flour (w.b.).

	Mass of ingredients (g) per 100 g of flour (w.b)				
Ingredients	LB-ABC	LF-LB	LF-LB-CO2	LB-CO2	RB-ABC
Flour	100	100	100	100	100
Butter	5.53	5.53	5.53	5.53	27.75
Icing sugar	31.85	31.85	31.85	31.85	-
Powder sugar	-	-	-	-	35.24
Water	20.77	20.77	20.77	20.77	8.72
ABC	1.00	-	-	-	0.44
SBC	0.64	-	-	0.64	0.44
SAPP 10	0.40	-	-	0.40	0.46
Milk powder	-	-	-	-	1.76
Salt	0.94	0.94	0.94	0.94	0.44
Glucose syrup	2.23	2.23	2.23	2.23	-
Total	**163.36**	**161.32**	**161.32**	**162.36**	**175.27**
Dough hydration (%)	**22.44**	**22.79**	**22.79**	**22.65**	**15.77**

(LB-ABC: laminated biscuit dough containing LAs mixed at atmospheric pressure; LF-LB: LAs-free laminated biscuit dough mixed at atmospheric pressure; LF-LB-CO2: laminated biscuit dough free from ABC, containing SBC and SAPP 10 with over-pressure CO_2 mixing; RB-ABC: rotary biscuit dough containing LAs mixed at atmospheric pressure).

3.1.3. Mixing procedure

3.1.3.1. Laminated biscuit dough mixing procedure

The laminated biscuit dough was prepared using the all-in method, meaning that mixing begins once all the ingredients are added to the mixer. Before mixing, glucose syrup was diluted in a small volume of water. ABC was also dissolved in a portion of water. The remaining water was combined with icing sugar and salt and thoroughly mixed to prevent agglomeration. All ingredients were then simultaneously introduced into the mixer (VMI, St-Hilaire-de-Loulay, France) with a single spiral blade.

A prototype batch mixer (VMI, St-Hilaire-de-Loulay, France) (Figure 3.1) with a bowl volume of 11 L and a specified capacity of up to 5 kg of dough was used for mixing. A water jacket that surrounds the mixing bowl regulates the dough's temperature while it is being mixed. The mixer is moving by rotating both the tool (agitator speed) and the satellites, while the bowl stays static.



Figure 3.1. Prototype batch mixer VMI (St-Hilaire-de-Loulay, France).

The mixer rotation speed was determined experimentally by selecting the speed that resulted in the highest void fraction in the dough and a final product density closest to 0.3 g/cm³. Experimental mixing conditions for laminated biscuit dough containing LAs are summarised in Table 3.2. It must be noted that for each test, a batch of 3 kg of dough was prepared, which is the minimum value needed for correct mixing.

Table 3.2. Experimental mixing conditions for laminated biscuit dough containing LAs at atmospheric pressure.

Experiment	Stage 1: Pre-mixing (min)	Stage 2: Mixing (min)	Stage 3: Kneading (min)	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage
E1	1	2	5	30	50,100,150	5,5,10
E2	1	2	8	30	50,100,150	5,5,10
E3	1	2	10	30	50,100,150	5,5,10
E4	1	2	15	30	50,100,150	5,5,10
E5	1	1	8	30	50,100,100	5,5,10

Experimental mixing conditions for laminated biscuit doughs with CO_2 over-pressure mixing are as follows (Table 3.3):

Table 3.3. Experimental mixing conditions for laminated biscuit dough with CO_2 over-pressure mixing.

Experiment	Stage 1: Pre-mixing (min) at -0.9 bar	Stage 2: Mixing (min) at 0.5 bar	Stage 3: Kneading (min) at 0.5 bar	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage
E1	1	2	10	30	50,100,100	5,5,10
E2	1	2	10	30	50,100,120	5,5,10
E3	1	2	10	30	50,100,180	5,5,10
E4	1	2	5	30	50,100,180	5,5,10
E5	1	2	10	30	50,100,180	5,5,10
E6	1	2	15	30	50,100,180	5,5,10
E7	1	2	10	25	50,100,180	5,5,10

During the mixing process, an infrared temperature sensor was used to monitor the dough temperature. The temperature was maintained at 30°C, except during certain CO_2 mixing tests, where it was lowered to 25°C to enhance the CO_2 solubility in water, since the dough contains a higher proportion of water compared to fat. Two methods were used in the CO_2 mixing tests: with gas flow and without gas flow (Figure 3.2). In the procedure without gas flow, during the pre-mixing stage, the valve was kept partially open at –0.9 bar (gauge pressure), and then fully closed during both the mixing

and kneading stages. As a result, there was no gas renewal in the headspace, and the gas present was a mixture of air and CO_2. In the procedure with gas flow, a vacuum of –0.9 bar (gauge pressure) was applied to the headspace during pre-mixing for one minute to remove ambient air and residual gases. To maintain sufficient oxygen renewal—since the dough consumes oxygen through solubilisation—a moderate airflow was introduced into the mixer's headspace during the mixing and kneading stages. Simultaneously, CO_2 was injected at a gauge pressure of 0.5 bar to enhance dough aeration.

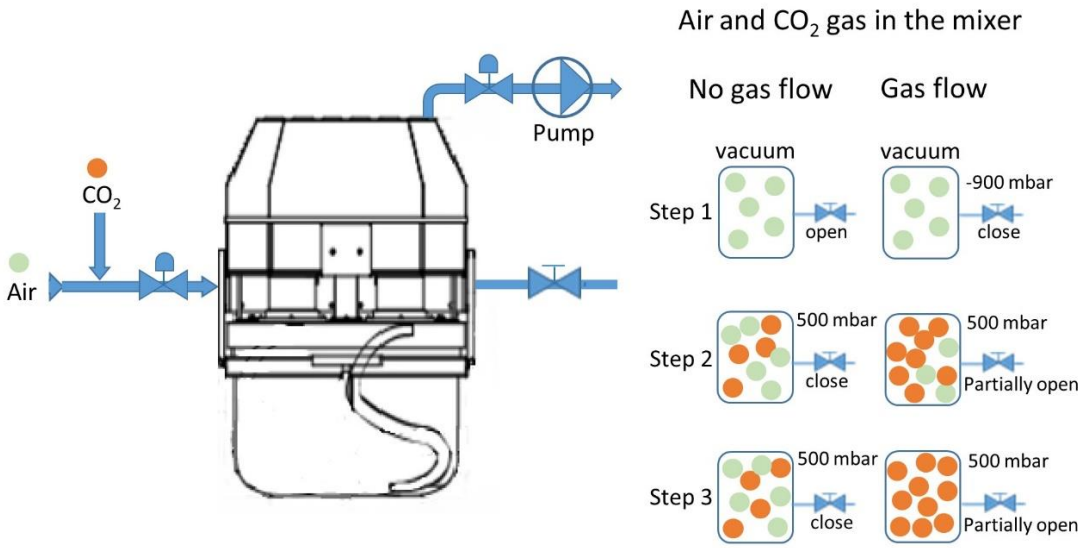

Figure 3.2. Air and CO_2 conditions in the VMI mixer at different mixing stages for CO_2 over-pressure mixing procedures, both with and without gas flow.

The LB-ABC and LF-LB biscuit dough mixing procedures are illustrated below (Figure 3.3).

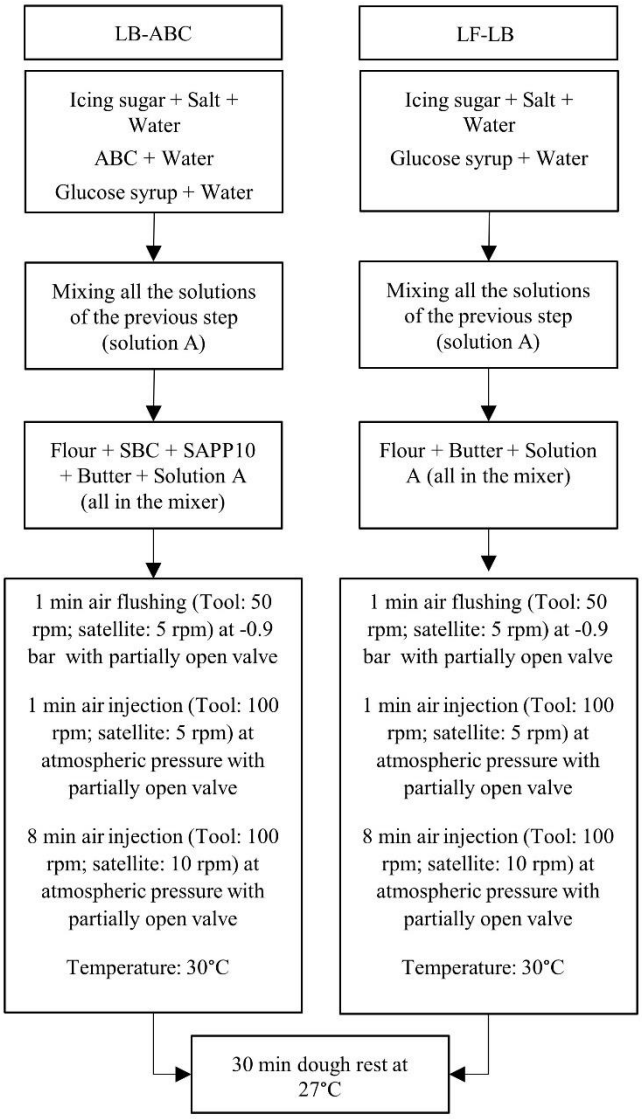

Figure 3.3. Laminated biscuit dough mixing procedure at atmospheric pressure; (LB-ABC: laminated biscuit dough containing LAs mixed at atmospheric pressure; LF-LB: LAs-free laminated biscuit dough mixed at atmospheric pressure).

The LF-LB-CO2 and LB-CO2 biscuit dough mixing procedures, with and without gas flow, are shown in Figure 3.4.

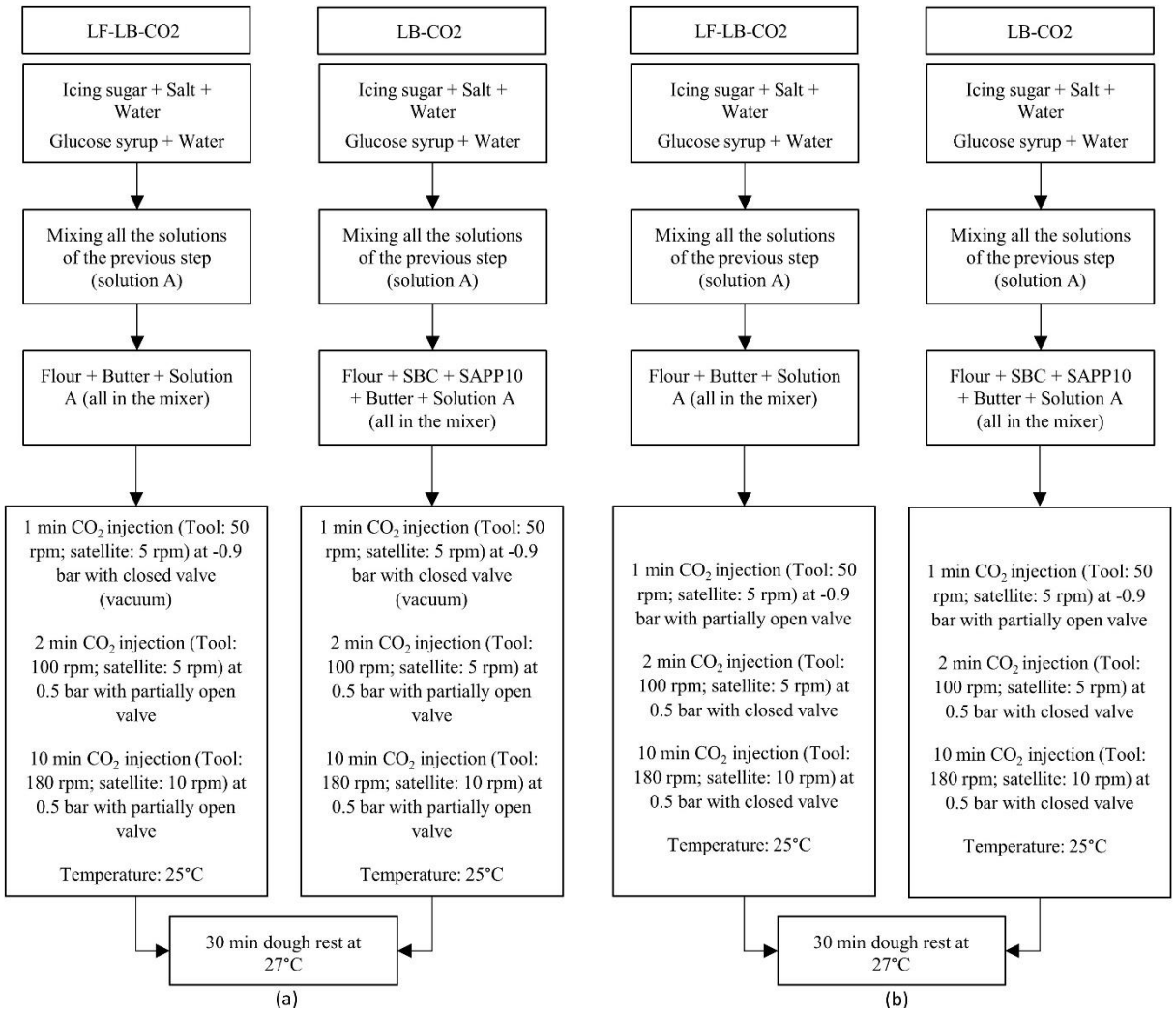

Figure 3.4 Laminated biscuit dough with CO_2 over-pressure mixing (a) with gas flow and (b) without gas flow; (LB-CO2: laminated biscuit dough free from ABC, containing SBC and SAPP 10 with over-pressure CO_2 mixing; LF-LB-CO2: laminated biscuit dough free from LAs with over-pressure CO_2 mixing).

3.1.3.2. Void fraction of gas in dough

3.1.3.2.1. Apparent density

The apparent density (bulk density) of dough (ρ_{dough}) was measured following the method proposed by researchers in 2017 (Sadot et al., 2017) with some modifications; this method relies on Archimedes' principle. In this procedure, dough is weighed in air, then submerged in a liquid of known density and weighed again; from the weight difference and the liquid's density, the dough's density can be determined. Canola oil was utilised as the immersion liquid.

To evaluate the ρ_{dough}, six dough samples of approximately 30 g each were collected from the mixed dough for each mixing condition. A 500 mL beaker was filled with canola oil and placed on a balance. A support was submerged below the oil's surface, and the balance was tared. Each dough sample was

then weighed outside the beaker to determine its mass ($m_{gas-free}$), representing the mass of the degassed (gas-free) dough. The volume of gas-free dough ($V_{gas-free}$) is:

$$\mathrm{V_{gas-free}} = \frac{m_{gas-free}}{\rho_{dough-degassed}} \qquad \textit{Eq. 3.1}$$

The dough sample was then placed on the support and submerged in the oil to the same depth as during the taring process. The resulting measurement, denoted as m_{buo}, corresponds to the buoyant force acting on the total volume of the dough sample (V_{dough}), which includes both the volume of gas-free dough ($V_{gas-free}$) and the volume of entrapped gas (V_{gas}).

$$\mathrm{m_{buo}} = \mathrm{V}_{dough} \cdot \rho_{oil} = V_{gas-free} \cdot \rho_{oil} + V_{gas} \cdot \rho_{oil} \qquad \textit{Eq. 3.2}$$

Therefore, V_{gas} and V_{dough} were calculated as follows:

$$\mathrm{V_{gas}} = \frac{\mathrm{m}_{buo} - V_{gas-free} \cdot \rho_{oil}}{\rho_{oil}} \qquad \textit{Eq. 3.3}$$

$$\rho_{\mathrm{dough}} = \frac{m_{gas-free}}{V_{gas-free} + V_{gas}} \qquad \textit{Eq. 3.4}$$

3.1.3.2.2. Real density

The real density (solid density) is the ratio of the mass of the matrix to its volume, excluding the gaseous fraction contained in the matrix. The gas-free dough density ($\rho_{dough-degassed}$) was calculated using the volume fraction (X_i^v) and the density (ρ_i) of all ingredients (Sahin & Şumnu, 2006) according to the following equation :

$$\rho_{dough-degassed} = \sum \rho_i \cdot X_i^v \qquad \textit{Eq. 3.5}$$

This equation does not take into account interactions between ingredients, as mentioned by Campbell et al. (1993) for bread dough. However, the inaccuracy of such an approach was considered minor because it affected all calculated values, which are therefore comparable.

3.1.3.2.3. Void fraction of gas in dough

The void fraction of gas in dough ($\emptyset_{dough}$) represents the volume fraction of gas contained in the dough. It is calculated as follows (Sadot et al., 2017):

$$\emptyset_{dough} = \frac{V_{gas}}{V_{gas-free} + V_{gas}} = 1 - \frac{\rho_{dough}}{\rho_{dough-degassed}} \qquad \textit{Eq. 3.6}$$

3.1.4. Rotary biscuit dough mixing procedure

The creaming method was used for rotary biscuit dough (RB-ABC). A KENWOOD mixer (France) equipped with a flat K-shape beater (Figure 3.5) was employed for dough mixing. The initial mixing procedure was developed by the CTCPA Technical Centre (France) and subsequently adapted to form dough using the forming machine. Thus, butter and sugar were introduced to the mixer and were mixed for 10 min at a speed of 106 rpm. ABC was dissolved in water and then added to the mixer. Salt and milk powder were also added at this phase. The mixing continued for 2 min at the same speed. In the last phase, SBC, SAPP10, and flour were introduced to the mixer and were mixed for 2 min at a speed of 106 rpm. Before forming, the dough rested for 15 min at ambient temperature.



Figure 3.5. KENWOOD (France) mixer equipped with a flat K-shape beater.

3.1.5. Forming procedure

3.1.5.1. Laminated biscuit forming procedure

Laminated biscuit dough was sheeted using a RONDO dough sheeter (RONDO, Germany) (Figure 3.6) after a 30-minute rest at 27°C. Lamination was carried out in five stages, with nips between the rolls of 10, 8, 6, 4, and 2 mm. The laminated dough was then shaped manually using a 60 × 60 mm rectangular cutter fitted with needles. Shaping took place 1 minute after lamination to minimise shrinkage. The biscuits were placed on an oven rack for baking. A total of 28 biscuits were baked at a time.



Figure 3.6. RONDO (Germany) dough sheeter.

3.1.5.2. Rotary biscuit forming procedure

Rotary moulded biscuit dough was shaped using a rotary moulder (PMP food machinery, Italy) (Figure 3.7) provided by the CTCPA Technical Centre at a speed of 4. Rotary biscuits are rectangular (50 × 39 mm) with a thickness of 2 mm. The biscuits were placed on an oven rack for baking, with 55 biscuits baked at a time.



Figure 3.7. Rotary moulding machine (PMP Food machinery, Italy).

3.1.6. Baking procedure

3.1.6.1.1. Optimal Conventional baking of laminated biscuits

Steam and humidity in the oven during biscuit baking can affect the properties of the final product. The purpose of injecting steam into the oven is to control the texture, crust formation, and overall quality of baked biscuits. The humidity in the oven was monitored using a temperature and humidity data logger HYGROX™-P4 (MCQUEEN CAIRNS, England) (Figure 3.8) to optimise the steam injection for baking.

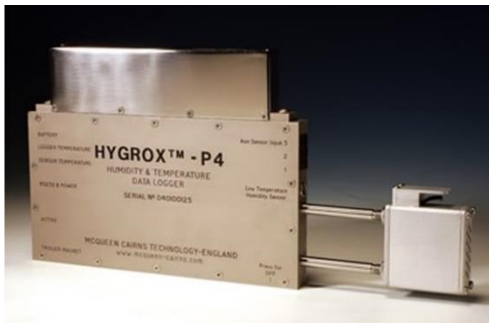

Figure 3.8. Temperature and humidity data logger HYGROX™-P4 (MCQUEEN CAIRNS, England).

For this test, the MIWE oven was preheated for 1 hr to reach the temperature set point. 28 laminated biscuits were placed on an oven tray and located in the MIWE oven, along with the HYGROX™-P4 data logger. Different baking conditions were applied (Table 3.4), and the information (time, oven temperature, specific humidity, relative humidity, and dew point) was recorded by the data logger. The quality of the biscuits (dimensions, water content, density, and surface appearance) was analysed after each baking. In the early stage, having the dew point close to the oven temperature is desirable to delay crust formation and improve biscuit thickness and texture. In the middle stage, a moderate dew point is favourable for setting, with a lower dew point in the final stage for drying. Excessive steam during baking results in biscuit rupture and the formation of surface cracks.

Table 3.4. Experimental plan for baking of laminated biscuits containing LAs.

Baking temperature (°C)	Baking duration	Level of injected water (mL) in the form of steam
260	5 min 30 S	140
260	5 min 30 S	280
240	9 min	140
240	9 min	180
240	9 min 30 S	140
240-250	9 min 30 s	140

3.1.6.1.2. Optimal conventional baking of rotary biscuits

Rotary moulded biscuits were baked in a deck oven (MIWE, Germany) at a temperature of 220°C for 6 min and 30 s. In each batch, 55 biscuits were placed on the oven tray for baking. After baking, they were allowed to cool at ambient temperature for 30 min. For additional analysis, they were placed in a sealed plastic bag once cooled.

3.1.7. Kinetics of mass loss during baking

To optimise the baking process, understanding the kinetics of mass loss during baking is crucial. The mass loss refers to the water lost from biscuit dough pieces, which occurs during baking as water evaporates. It was measured by placing 12 laminated biscuits on the oven tray, which was connected to a hook and a modular acquisition system (AOiP, France) that recorded the mass loss in the MIWE oven (Figure 3.9).



Oven tray connected to a data acquisition system



Modular data acquisition AOiP

Figure 3.9. Mass loss measurement system for biscuits during baking designed at the GEPEA. The oven tray was attached to a balance positioned above the oven, allowing the mass of the biscuits to be recorded during baking.

3.1.8. Thermophysical properties: freezable water

A Multi-Cell DSC (TA Instruments-Waters, US) was used to evaluate the enthalpy of freezable water in the dough. Around 150 mg of dough was hermetically sealed in aluminum cells. The samples and reference were then placed inside the calorimeter. The thermal program includes an equilibration phase at 22°C for 1 min, followed by a cooling phase from 20°C to -40°C at a rate of 5°C/min. The sample was then held at -40°C for 5 min and then heated to 20°C at the same rate. The enthalpy of fusion was then determined using the software (TA Instruments, US) and expressed in J/g (d.b.).

The amount of water that can be frozen ($m_{W-freezable}$) was obtained from the ratio between the energy of fusion (Q_f) of the water solidified during freezing and the latent heat of fusion (L_f) of pure water, which is around 334 J/g :

$$m_{W-freezable} = \frac{Q_f}{L_f} \qquad \textit{Eq. 3.7}$$

The amount of CO_2 dissolved in dough through mixing can be estimated by analysing the $m_{W-freezable}$. This value is expressed in g freezable water/g biscuit dough.

3.2. Impact of removing leavening agents (LAs) from laminated biscuits

Once the optimal dough mixing and baking conditions were determined for laminated biscuit dough containing LAs (LB-ABC), with reduced LAs (LB-CO2), and free from LAs (LF-LB-CO2), the characteristics of both the dough and the baked biscuits were measured.

3.2.1. Characterisation methods

3.2.1.1. Dough characteristics

3.2.1.1.1. pH

The pH of biscuit dough was measured at 25°C with a solid electrode (WTW 3110, Xylem Analytics, Germany).

3.2.1.1.2. Void fraction of gas in dough

The void fraction of gas in the laminated biscuit dough was measured according to *Eq. 4.2- Eq. 4.2*, as described in Section 3.1.3.2 of the Materials and Methods.

3.2.1.2. Biscuit characteristics

3.2.1.2.1. Porosity

- Apparent density

The apparent density of biscuits (ρ_{bis}) is defined as the ratio between the total mass (m_{baked}) and the total volume of biscuits, including any trapped gas. It was calculated by measuring the thickness, length, and width of eight baked biscuits with a digital calliper (Mitutoyo, Japan) and weighing them. Thickness was defined as the average of the thickness at three points.

$$\rho_{bis} = \frac{m_{baked}}{length \cdot width \cdot thickness} \qquad Eq.\ 3.8$$

- Real density

The real density of biscuits ($\rho_{solid-bis}$) without pores was measured using a helium pycnometer (Accupyc 1330, Micromeritics, US), 10 times for each sample.

- Porosity

Biscuit porosity ($\emptyset_{bis}$) was calculated according to the following equation:

$$\emptyset_{bis} = 1 - \frac{\rho_{bis}}{\rho_{solid-bis}} \qquad \textit{Eq. 3.9}$$

Where ρ_{bis} is the apparent density of biscuit (pores included) and $\rho_{solid-bis}$ is the real density of the pure biscuit without pores. The porosity value is expressed as a percentage.

3.2.1.2.2. Water content

The water content of the biscuits was determined following the AACC method 44–15.02. A sample of 3 g (w.b.) of powdered biscuit was placed in an aluminium cup and dried in a drying cabinet at 105°C for 24h. After drying, the sample was placed in a desiccator for 10 min before being weighed. Measurements were performed in triplicate.

3.2.1.2.3. Colour

The colour of five biscuits was assessed 24h after baking with a colourimeter (Konica Minolta, France), targeting the centres and edges of both the top and bottom surfaces. The colour measurements were based on the CIE system parameters: L^* (lightness), a^* (redness), and b^* (yellowness) (Figure 3.10). The difference in colour (ΔE) between the control (with notation 1) and reformulated biscuits (with notation 2) was determined using equation (*Eq. 3.10*). The CIE L^* a^* b^* representation model, developed in 1976 by the International Commission on Illumination (CIE), covers the entire visible spectrum and codifies each colour using three parameters: L^* ranging from 0 (black) to 100 (white), a^* corresponding to the range (-) green – red (+) and b^* representing the hue on the axis (-) blue – yellow (+). On this a^*b^* scale, the value 0 corresponds to the colour grey.

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad \textit{Eq. 3.10}$$

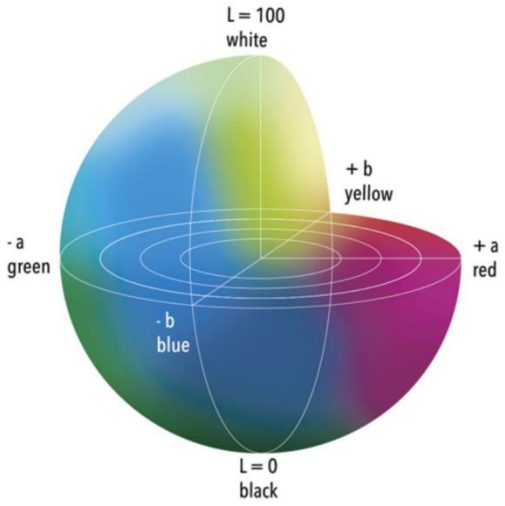

*Figure 3.10. CIE L*a*b* colour space.*

3.2.1.2.4. Texture

Hardness analysis of biscuits was conducted using a texture analyser (AMETEK-TA, US) coupled with a load of 1 kN. The three-point bending test was applied to quantify the hardness. The measurement pre-test speed was 1 mm/s, the test speed was 5 mm/s, and the distance was 10 mm. The span between the supports was 35 mm. The force-displacement plots were analysed for peak breaking force (N). Textural attributes were measured in five samples.

3.3. Microwave baking of biscuits as an alternative to conventional baking

Once laminated and rotary biscuit doughs containing LAs (LB-ABC and RB-ABC) were prepared, a microwave baking process was applied to them (LB-ABC-MW, RB-ABC-MW). Laboratory-size biscuits (3 cm in diameter) were baked using a solid-state microwave oven at the GEPEA laboratory. Full-size biscuits were baked at the CTCPA Technical Centre in Avignon, France. Additionally, some biscuits underwent a second baking, either infrared or conventional baking (LB-ABC-MW-CB, RB-ABC-MW-CB).

3.3.1. Microwave baking

Microwave coupling with dielectric materials depends on various factors, including cavity size, dielectric properties, frequency, and sample geometry. In the case of a multi-mode microwave cavity, such as that found in a domestic microwave oven, the power transmitted from the generator to the cavity can be absorbed by the sample, reflected to the generator, or lost through dissipation in connections, cables, and metallic walls. To optimise microwave heating processes, experimental characterisation of the dielectric properties of food materials is essential, as these parameters are critical for evaluating and understanding the interaction between microwaves and polar molecules (Frabetti et al., 2023).

3.3.1.1. Dielectric properties of non-baked biscuits

The dielectric properties of biscuit doughs at microwave frequencies were measured using a dielectric assessment kit designed for thin layers (DAK-TL2, Schmid & Partner Engineering AG, Switzerland). The measurement principle is based on an open-ended coaxial probe coupled with an integrated electronic system, which allows automated control of the applied mechanical force and precise determination of the sample thickness. Dielectric parameters are extracted from the reflection coefficient measured at the probe flange, accounting for both the probe geometry and the measured thickness of the sample.

In the DAK-TL2 system (configuration DAK-3.5-TL2: 200 MHz–20 GHz), the probe assembly is mounted on a base unit, while a fully automated, software-controlled sample stage enables to perform the dielectric properties measurement of the sample placed in contact with the probe (Figure 3.11). The platform simultaneously measures the sample thickness with micrometer-scale precision. In addition, the mechanical force applied to the top surface of the sample can be finely regulated to ensure optimal contact with the probe, thereby improving repeatability and measurement reliability.

The DAK-TL2 device was connected to a vector network analyzer (VNA, ENA 5062A, Agilent Technologies, Malaysia) via a coaxial cable. Calibration was performed through the software using

standard procedures (open, short circuit, and load with a material of known dielectric properties). The dough sample (a round disc with a diameter of 3 cm and a thickness of 2 mm) was placed between the probe and the support plate (Figure 3.11). Measurements were carried at a frequency of 2.45 GHz with an applied mechanical force of 30 N. The probe-sample interface may be impacted by differences in surface texture and compliance in soft heterogeneous materials like dough. By applying a controlled load measurement, stability is increased and air gaps are reduced. The extracted dielectric parameters at 2.45 GHz include the relative dielectric constant (ε_r'), the relative dielectric loss factor (ε_r''), and the dissipation factor, tan δ.

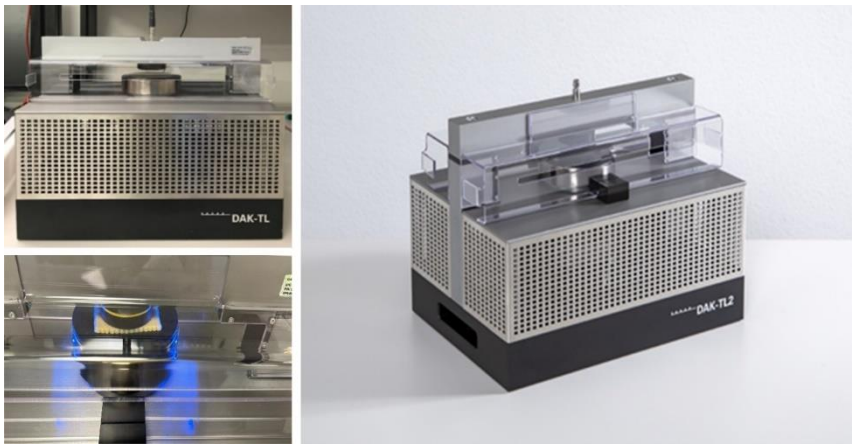

Figure 3.11. Dielectric probe DAK-TL2 (Schmid & Partner Engineering AG, Switzerland) for measuring the dielectric properties of biscuit doughs.

3.3.1.2. Microwave cavity characterisation

The microwave cavity is based on a modified household microwave oven (Figure 3.12). The magnetron was removed from the initial device and replaced with an antenna to connect the microwave cavity to the microwave power source.

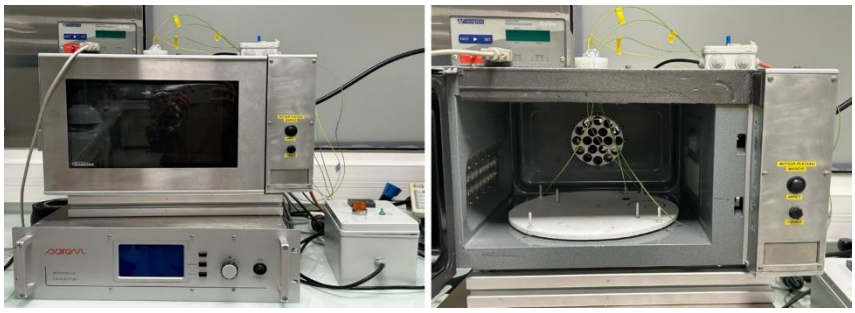

Figure 3.12. Modified household microwave oven connected to a 200 W solid-state generator SAIREM (France).

To monitor power transfer between the source and the load, the microwave cavity was connected to a vector network analyser (VNA, S5045, Copper Mountain Technologies, US) (Figure 3.13). The VNA is first calibrated using a standardized procedure (open, short and load) from a mechanical calibration

kit (85519A, Keysight Technologies, US). Once the VNA calibration is done, a one-port microwave cavity is considered (port 1 of the VNA is only used). Based on these operating conditions, the VNA measures the S11 parameters defined as follows:

$$S11\ (dB) = 10 \cdot \log\left(\frac{P_{ref}}{P_{inc}}\right) \qquad \textit{Eq. 3.11}$$

Where P_{ref} and P_{inc} are reflected power and incident power, respectively. P_{ref}/P_{inc} represents the reflection coefficient.



Figure 3.13. Vector network analyser S5045 (Copper Mountain Technologies, US) for measuring the electromagnetic properties of materials and systems.

Several shapes and sizes for microwave heating of biscuits were initially tested experimentally. Preliminary experiments indicated that microwave heating was strongly heterogeneous with rectangular biscuits (especially at the corner side). Microwave baking of round biscuits with a 3 cm diameter produced a more even baking process. Consequently, this particular shape and size were selected for baking biscuits. Microwave interaction with food depends heavily on shape. In spherical and cylindrical items (20–60 mm diameter), waves tend to focus more uniformly inside the product, while in rectangular shapes, the electric field concentrates at the corners, leading to localised overheating (Cao, 2012).

To determine the optimal position for baking biscuits inside the microwave cavity, experiments were performed using unbaked biscuits. Each biscuit (laminated or rotary) was placed at different locations on a Teflon® plate, and the reflected power (P_{ref})—the percentage of microwave energy that does not enter the sample but is instead reflected to the source—was measured. These measurements were obtained using a vector network analyzer (S5045, Copper Mountain Technologies, US) connected to the antenna. Depending on the dielectric properties of the biscuits (laminated or rotary), their optimal position may vary. The best geometrical positions on the tray were selected, where the reflected microwave power was minimal. For characterising electromagnetic field interaction with dielectric materials inside MW cavities, the quality factor (Q-factor) is often used and represents the ratio between the energy stored in the system (from the input microwave signal) and the energy lost per cycle of oscillation (Combes, 1996).

$$Q\text{-}factor = 2\pi \cdot \frac{Energy\ stored\ in\ the\ system}{Dissipated\ energy\ per\ cycle} \qquad Eq.\ 3.12$$

To enhance microwave power absorption around the sample region, a dielectric resonator—typically a cylindrical ceramic with high permittivity and a very low loss tangent—is placed inside the cavity next to the dielectric sample. The resonator strongly confines the electromagnetic field, concentrating the stored energy in a region with minimal losses. Because of its intrinsically high Q-factor, the resonator redistributes the electromagnetic field within the microwave cavity, directing it into areas of low dielectric loss. As a result, for high-quality resonators and samples with low dielectric losses, the reflected microwave power is significantly reduced due to the improved Q-factor of the cavity.

For biscuits with a low loss tangent, such as laminated biscuit dough (indicating a high Q-factor), a cylindrical ceramic dielectric resonator (Temex Ceramics, 2.3 cm in diameter and 1 cm in height) was placed next to the dough sample to increase the overall Q-factor of the cavity and reduce the microwave reflection coefficient.

3.3.1.3. Microwave baking procedure

Both laminated and rotary biscuits were baked in a multimode microwave cavity connected to a 200 W solid-state microwave generator (GLS200, SAIREM, France) (Figure 3.14) operating at a frequency of 2.45 GHz.



Figure 3.14. 200 W solid-state microwave generator (GLS200, SAIREM, France).

The optimal sample position was determined by measuring the P_{ref} while emitting power, P_{inc}, from the microwave generator. Laminated and rotary biscuits—one at a time—were carefully placed at the optimal position on top of a PTFE support plate (Figure 3.15), corresponding to the region where reflected microwave power was minimum. A ceramic resonator was also placed next to the dough sample during microwave processing. During the treatment, air inside the cavity was slightly extracted using a fan positioned at the back of the microwave cavity. Temperature measurements were taken at four different locations within the biscuit during microwave processing, using optical fibres connected to a data logger (Neoptix Reflex®, Canada) (Figure 3.15).

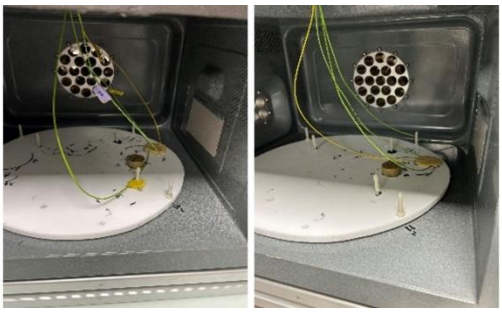

Figure 3.15. Optical fibres connected to a data logger (Neoptix Reflex®, Canada) placed within a biscuit at the optimal position in the microwave cavity.

Incident microwave power of 30 W was applied for 15 min to laminated biscuits and 10 min to rotary biscuits. The reflected microwave power was monitored on the microwave generator throughout the processing. The mass loss of the biscuits was measured by weighing them before and after baking in the microwave oven. Since the microwave-baked biscuits appeared pale at the end of the process, they were placed in a modified oven (Ramalhos, France) equipped with 5 infrared lamps (Figure 3.16) for 30 s at 100% power to improve the crust colour. These infrared lamps are separated into three zones, where the power can be adjusted between 0% and 100%. Zone 1 has only one lamp and is located near the oven opening. Zone 2, located in the centre of the oven, and zone 3, located at the back of the oven, have two lamps. The infrared lamps have a power of 2 kW each when a voltage of 400V passes through them.



Figure 3.16. Ramalhos oven equipped with five infrared lamps.

3.3.1.4. Energy consumption in microwave baking of biscuits

3.3.1.4.1. Evaluation of energy efficiency of the microwave oven

To assess the energy consumption and efficiency of microwave baking, it is important to consider that microwave ovens are not 100% efficient in converting electrical energy into heat. In this experiment, a known quantity of water was placed at the optimal spot in the microwave cavity, and the energy

required to heat the water to a specific temperature was measured. Thus, 10, 20, 30, 40, and 50 g of water were poured into a Teflon container with a lid, which featured a small hole for inserting optical fibres. Four optical fibres were immersed in the water to monitor temperature changes (ASTM International, 2019).



Figure 3.17. Placing water in the microwave cavity for evaluating the energy efficiency of the microwave.

The initial water temperature was recorded (~22°C). A microwave power of 40 W was applied, and the temperature evolution was tracked and recorded until the water reached approximately 50°C. The average temperature was measured after stirring the water immediately in the Teflon container. The energy transferred to the water,Q, was then calculated using the following equation:

$$Q = m \cdot C_p \cdot \Delta T \qquad \textit{Eq. 3.13}$$

where m is the initial mass in g, C_p is the specific heat capacity in J/g °C (4.184), ΔT is the temperature change (between the final and initial temperature) in °C, and Q is the energy transferred to the water in J.

The effective power (P_{eff}) transmitted to the water was calculated by dividing the energy absorbed by the time during which the energy was supplied.

$$P_{eff} = Q/t \qquad \textit{Eq. 3.14}$$

The efficiency of the microwave was computed as the ratio of absorbed power (P_{eff}) to the incident power (P_{inc}):

$$Efficiency\ (\%) = 100 \cdot \frac{P_{eff}}{P_{inc}} \qquad \textit{Eq. 3.15}$$

3.3.1.4.2. Baking energy of biscuits

For measuring the energy consumption for baking biscuits, the specific heat capacities of laminated ($C_p^{laminated}$) and rotary (C_p^{rotary}) biscuit doughs were first determined over a temperature range of 20°C to 60°C using a Multi-Cell Differential Scanning (DSC) (TA instruments-Waters, US). The dough pieces were then placed at the optimal position in the microwave cavity, with the ceramic resonator positioned next to them. Optical fibres were inserted into the biscuits to monitor the temperature. The dough pieces were heated until the temperature reached 60°C, with the average temperature taken as the final value. The energy transferred to the dough pieces as they were heated to 60°C was calculated using Equation (*Eq. 3.13*). The P_{eff}, and $Efficiency\ (\%)$ were determined using Equations (*Eq. 3.14*) and (*Eq. 3.15*), respectively. P_{emit} was calculated as the difference between the incident power (P_{inc}) and the P_{ref} in the microwave.

$$P_{emit} = P_{inc} - P_{ref} \qquad Eq.\ 3.16$$

The mass loss was measured by weighing the dough pieces before and after heating.

3.4. Impact of biscuit formulation and baking on acrylamide (AA) formation

After preparation of conventionally-baked laminated and rotary biscuits (LB-ABC-CB and RB-ABC-CB), laminated biscuits with reduced LAs (LB-CO2-CB), and microwave and combined microwave-convetional baked laminated and rotary biscuits (LB-ABC-MW, RB-ABC-MW, LB-ABC-MW-CB, and RB-ABC-MW-CB), the AA content of all samples was analysed. The AA analysis was carried out at the International Hellenic University (IHU) in Thessaloniki, Greece, as part of a three-month international mobility project, under the supervision of Prof. Maria Papageorgiou and in collaboration with Dr. Kali Kotsiou.

3.4.1. Biscuit preparation

LB-ABC-CB, RB-ABC-CB, and LB-CO2-CB biscuits were mixed, formed, and baked according to the procedures described in Sections 3.1.2, 3.1.3, 3.1.4, 3.1.5, and 3.1.6 of the Materials and Methods. For LB-ABC-MW, RB-ABC-MW, LB-ABC-MW-CB, and RB-ABC-MW-CB biscuits, the baking step was carried out at the CTCPA Technical Centre in Avignon, France, using an industrial-scale continuous microwave oven.

Both laminated and rotary biscuits were made at the GEPEA laboratory and were frozen at -18°C, 4 weeks before the baking tests. For the baking tests, the dough pieces were left on a baking tray for thawing for around 1h, even though the amount of frozen water was very low. Then dough pieces were installed on the baking support, which was made from BBQ baking net coated with PFAS coating (Teflon®, Dupont). The baking net was installed on the conveyor belt of the SAIREM prototype microwave oven (France), which is used for the industry baking tests. The microwave oven was equipped with 8 magnetrons, operating at 2450 MHz. Each magnetron had a nominal power of 2 kW (Figure 3.18).

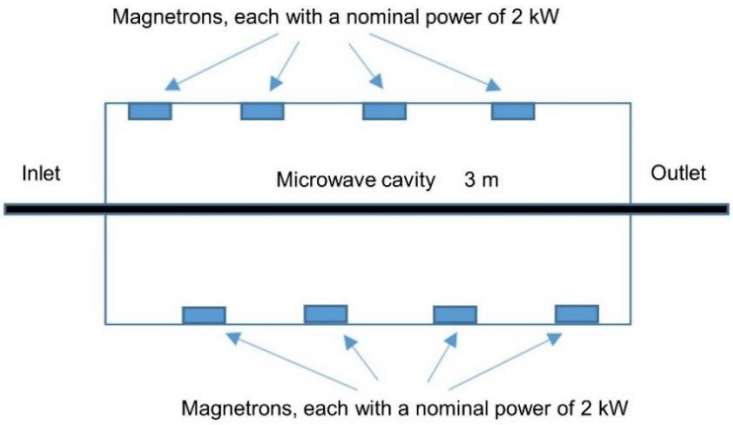

Figure 3.18. SAIREM prototype microwave oven schema used for industrial baking tests, equipped with 8 magnetrons with a nominal power of 2 kW each.

The power emitted by each magnetron (PXT and PXB) is summarised in Table 3.5 for both types of biscuits. A total power of 3.4 kW and 2.8 kW was applied to laminated and rotary biscuits, respectively. The conveyor speed and residence time were 37 cm/min and 8 min, respectively. During baking, changes in the temperature of the biscuits were monitored using optical fibres. Since microwave baking is not 100% efficient and only part of the power is absorbed by the food in the cavity, the reflected power (RP) was measured as well. In case of combined microwave-conventional baking (LB-ABC-MW-CB and RB-ABC-MW-CB biscuits), microwave baking was followed by 2 min of baking in a conventional oven (AKZ96490 IX, Whirlpool, France) at 250°C.

Table 3.5. Power values and specific energy for the baking of laminated and rotary biscuits; (SE: specific energy in MJ/kg dough; PXT: power of the top magnetron; PXB: power of the bottom magnetron; PT: total power; RP: reflected power).

Type of dough	P1T	P1B	P2T	P2B	P3T	P3B	P4T	P4B	PT	RP1	RP2	PT-PR	SE
Laminated	0.3	0.3	0.3	0.3	0.5	0.5	0.6	0.6	3.4	0.14	0.29	2.97	1.41
Rotary	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	2.8	0.1	0.2	2.5	1.18

3.4.2. Characterisation methods

The characteristics of the doughs and baked biscuits were determined according to the procedure described in Section 3.2.1 of the Materials and Methods, except for colour.

The surface colour of the biscuits was assessed using a MetaVue colourimeter (X-rite, US), following the CIELAB colour space system. This system quantifies colour using three parameters: L* (lightness), ranging from 0 (black) to 100 (white); a*, indicating the red–green axis (negative values denote green, positive values indicate red); and b*, representing the blue–yellow axis (negative values for blue, positive values for yellow). For each type of biscuit, three individual biscuits were randomly selected. On each biscuit, colour readings were taken at five distinct surface points**,** and the mean value was calculated. The instrument was routinely calibrated to ensure measurement accuracy throughout the analysis. The Browning index (BI) was then calculated based on the CIE values of L* a* b* according to the following equations (Masciola et al., 2025):

$$BI = \frac{(-0.31) \cdot 100}{0.17} \quad \text{Eq. 3.17}$$

Where X is:

$$X = \frac{a^* + 1.75 \cdot L^*}{5.645 \cdot L^* + a^* - 3.012 \cdot b^*} \quad \text{Eq. 3.18}$$

3.4.3. Acrylamide (AA) analysis

3.4.3.1. Chemicals for analysis

AA powder (≥ 99%) was obtained from Sigma-Aldrich (Germany), and acrylamide-2,3,3-d_3 powder (> 98%) was obtained from C/D/N Isotopes Inc. (Canada). Water and methanol (HPLC grade) were supplied by Chem-Lab NV (Belgium). Carrez I (potassium hexacyanoferrate (II) trihydrate) and Carrez II (Zinc Sulfate Heptahydrate) were purchased from Fluka Biochemika (Switzerland). Oasis MCX cartridges (1 cc) (Waters™, Ireland) were used for AA extract purification.

3.4.3.2. Acrylamide (AA) extraction

AA analysis of the biscuits was performed after a triple-stage extraction as described by Zili´c et al. (2024) with some modifications. AA (molecular weight 72 Da, CH_2=CH–CO–NH_2) is a small, highly water-soluble molecule; therefore, lightly acidified water was used as the extraction medium. Seven biscuits were finely ground in a grinder prior to extraction. A 10 mM formic acid solution containing 20 ng/mL of d_3-acrylamide, used as an internal standard, was prepared as the extraction solvent. In the first extraction step, 0.5 mL Carrez I, 0.5 mL Carrez II, and 9 mL of extraction solution were added to 1 g of finely ground sample and vortexed for 3 min. The supernatant was then removed and collected in a tube after centrifugation for 10 min at 8000 rpm at 4°C. In the second extraction step, 5 mL of

extraction solution was added to the pellet, vortexed for 3 min, and then centrifuged at 8000 rpm at 4°C for 10 min to separate the supernatant, which was collected in a tube. The third extraction followed the same stages as the second. All supernatants were combined in a tube, sealed, and stored at 4°C until analysis.

3.4.3.3. Extract purification by solid phase extraction (SPE)

The Oasis MCX cartridge (1 cc) was conditioned for activation of the sorbent functional groups by the successive addition of 1 mL methanol (HPLC grade) followed by 1 mL water (HPLC grade) at a flow rate of approximately 1 drop/s under a vacuum of 375 mmHg. The supernatant of the centrifuged extract was then passed through the conditioned Oasis MCX cartridge. For the clean-up of extracts in AA analysis, mixed-mode cation-exchange cartridges are preferred because positively charged amino acids present in the matrix are retained by the sorbent material. The first 8 drops of the eluate were discarded, and the remaining portion was collected into an Eppendorf tube. The collected extract was filtered through a 0.22 µm nylon filter and transferred into vials prior to LC–MS/MS analysis.

3.4.3.4. LC-MS/MS analysis of acrylamide (AA)

The clear extracts transferred into vials were analysed using an Agilent 1260 LC system coupled to an Agilent 6420 Triple Quadrupole detector (Agilent Technologies, US). Chromatographic separation was performed on a Zorbax XDB C18 column (2.1 × 150 mm, 3.5 µm; Agilent, Waltham, MA, US) using 10 mM formic acid in water as the mobile phase at a flow rate of 0.3 mL/min and a column temperature of 25°C. The injection volume was 5 µL. Due to its low molecular weight (72 Da) and high polarity, AA elutes very early in reversed-phase chromatography; therefore, careful control of chromatographic conditions was required. The electrospray ionization (ESI) source was operated with a gas temperature of 350°C, capillary voltage of 2000 V, gas flow of 11 L/min, and nebuliser pressure of 60 psi. Data acquisition was performed in multiple reaction monitoring (MRM) mode. The precursor ion $[M + H]^+$ at m/z 72 was fragmented to product ions at m/z 55 (quantifier, collision energy 9 V), m/z 44 (qualifier, CE 12 V), and m/z 27 (qualifier, CE 24 V). Deuterated AA (d_3-acrylamide) was used as the internal standard, with a precursor ion at m/z 75 and a product ion at m/z 58. The dwell time for all MRM transitions was set to 0.2 s. Quantification of AA was performed using a calibration curve (1–50 ng/mL) based on the peak area ratio of AA to the internal standard d_3-acrylamide. Two replicate measurements were conducted for each sample, and results were expressed as ng per g of sample (d.b.). The LC–MS/MS system, data acquisition, and processing were managed using MassHunter Workstation Software (Version B.06.00, Agilent Technologies, US).

3.4.4. Sensory analysis

LB-ABC-CB, RB-ABC-CB, LB-CO2-CB, LB-ABC-MW-CB, and RB-ABC-MW-CB were included in the sensory analysis. LB-ABC-MW and RB-ABC-MW were excluded due to an insufficient number of samples. These samples were studied to determine the influence of reducing LAs and baking method on overall biscuit enjoyment and different biscuit attributes.

3.4.4.1. General conditions

3.4.4.1.1. Ethical statement

The sensory tests were conducted in accordance with the Declaration of Helsinki and with the approval of Inserm's ethical evaluation committee/Institutional Review Board (CEEI/IRB00003888).

3.4.4.1.2. Sensory analysis laboratory

The tests took place in the sensory analysis room at Oniris, which complies with the NF EN ISO 8589 (2010) standard. The room is equipped with 30 individual booths, with controlled temperature and lighting conditions.

3.4.4.1.3. Panellists

Panellists were recruited voluntarily from Oniris school, so the panel consisted of Oniris students and staff. Participants were informed about the study's purpose through an information sheet. Participants with known allergies or intolerances to the biscuit ingredients were excluded from the study, and those who agreed to participate signed a consent form after reading it.

3.4.4.1.4. Experimental conditions

The samples were prepared in advance for organisational purposes and were stored in sealed bags and kept in a dark place at ambient temperature. The samples were presented in identical cardboard boxes, each containing one biscuit from a specific recipe and coded with a three-digit random number. At the beginning of the session, each panellist received a tablet with a questionnaire developed using FIZZ software (BIOSYSTEMES, France). Panellists were also provided with a tray containing the biscuit samples and a cup of water (Evian, France). The sequence in which the biscuits were evaluated was randomised and balanced across panellists using FIZZ software (BIOSYSTEMES, France) to minimise order effects. Panellists were instructed to rinse their mouths between each sample.

3.4.4.2. Study of the influence of biscuit formulation and baking method on panellists' perception

69 panellists were carried out hedonic and Check-All-That-Apply (CATA) sensory analysis tests. The composition of the panel is shown below (Figure 3.19):

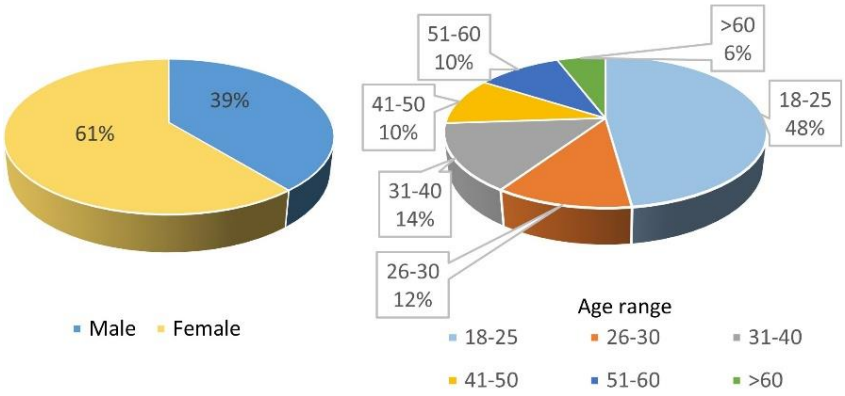

Figure 3.19. Composition of panel used for the hedonic and CATA tests on conventional and microwave-baked laminated and rotary biscuits (n = 69).

3.4.4.2.1. Hedonic test

Overall liking was evaluated using a 9-point hedonic scale, where a rating of 1 indicated "extremely dislike," 5 represented "neither like nor dislike," and 9 signified "extremely like."

3.4.4.2.2. Check-All-That-Apply (CATA) test

For the CATA test, the panellists were given a list of 40 sensory attributes (Figure 3.20) and instructed to mark all the attributes they perceived in the product being assessed. The attributes were selected based on the literature and a discussion among three individuals with a background in food science.

Yellow interior	Cereal flavour/ odour	Crispy
White interior	Caramel flavour/ odour	Grainy
Thin	Salty	Sandy
Thick	Aftertaste	Brittle
Presence of spots	Sweet	
Smooth surface	Cake batter flavour/ odour	
Golden colour	Baking powder flavour/ odour	
Brown colour	Flour flavour/ odour	
Homogeneous appearance	Acid	
Heterogeneous appearance	Hard	
Bumpy surface	Doughy	
Brillant	Dry	
Matte	Melt-in-the-mouth	
Buttery flavour/odour	Absorbs saliva	
Vanilla flavour/odour	Crumbly	
Toasted flavour/ odour	Crunchy	
Fatty sensation	Soft	
Milky flavour/odour	Floury	

Figure 3.20. List of CATA sensory analysis attributes.

3.5. *In vitro* starch digestibility and estimated glycaemic index (eGI)

In this part of the Materials and Methods, *in vitro* starch digestibility and eGI measurement of conventionally-baked laminated and rotary biscuits (LB-ABC-CB and RB-ABC-CB), LAs-free laminated biscuit (LB-CO2-CB), and combined microwave-convetional baked laminated and rotary biscuits (LB-ABC-MW-CB and RB-ABC-MW-CB) are discussed.

3.5.1. Estimated glycaemic index (eGI)

The *in vitro* procedure was based on the method proposed by Goni and Saura-Calixto (Goni & Saura-Calixto, 1997), which involves an *in vitro* starch hydrolysis procedure to estimate the GI, with some modifications.

3.5.1.1. Resistant (RS) and digestible starch (DS)

An external laboratory measured the RS and the digestible starch (DS) content of the samples using spectrophotometry and reported them per 100 g of sample.

3.5.1.2. Total starch (TS)

The total starch (TS) was calculated as the sum of the RS and the DS.

3.5.1.3. *In vitro* starch digestion

3.5.1.3.1. Preparation of buffers and enzymes

- HCl-KCl (0.2M, pH=1.5)

The 0.2M HCl-KCl solution was made by dissolving 14.9 g of KCl anhydrous (ref. 131494.1211 PanReac AppliChem, ITW Reagents, Spain/Germany) in distilled water in a 1 L volumetric flask. The pH was carefully adjusted to 1.5 by gradually adding 1M HCl (ref GH21001 Reagecon™, Fisher Scientific, Ireland), and the volume was then adjusted to 1 L with distilled water. This buffer is conserved at 4°C.

- Tris-maleate (0.1M, pH=6.9)

The 0.1M Tris-maleate buffer was prepared in a 1 L volumetric flask by dissolving 23.72 g of Trizma® maleate (ref T3128-250G Sigma-Aldrich, USA) in about 800 mL of distilled water. The pH was set to the desired value using 1M HCl (ref GH21001 Reagecon™, Fisher Scientific, Ireland). The solution was then adjusted to a final volume of 1 L with distilled water. Tris-maleate buffer is kept at 4°C.

- Sodium acetate (0.4M, pH = 4.75)

This buffer was prepared by dissolving 3.283 g of $C_2H_3NaO_2$ anhydrous (ref 131633.1210 PanReac AppliChem, ITW Reagents, Spain/Germany) in approximately 70 mL of distilled water. The pH was adjusted to 4.75 by adding 1M HCl (ref GH21001 Reagecon™, Fisher Scientific, Ireland), and the final volume was adjusted to 100 mL with distilled water. This buffer can be prepared in advance and stored at 4°C for up to one month.

- Pepsin solution

For this solution, 80 mg of pepsin (ref P6887-1G Sigma-Aldrich, US) was dissolved in 800 µL HCl-KCl (0.2M, pH=1.5) buffer. This solution must be prepared on the day of the experiment, a few minutes before utilisation, and kept on ice to maintain enzyme activity.

- α-amylase solution

30 mg of pancreatic α-amylase (ref A3176-1MU Sigma-Aldrich, US) was dissolved in 30 mL of Tris-maleate buffer. The solution was then diluted 12.6-fold to a final volume of 20 mL by removing 1.59 mL of the initial solution and adjusting the volume to 20 mL with Tris-maleate buffer.

- Amyloglucosidase

Amyloglucosidase was from Roche Diagnostics GmbH (ref 10102857001, Germany) and was used directly in the test without any prior preparation.

- GOPOD D-glucose assay kit (K-GLUC)

This kit was purchased from Megazyme (Ireland) and contains the following components: GOPOD reagent buffer (50 mL, pH 7.4, with p-hydroxybenzoic acid and sodium azide (0.09% w/v)), GOPOD reagent enzymes (glucose oxidase, peroxidase, and 4-aminoantipyrine), and a D-glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.

- GOPOD reagent

The contents of one bottle of GOPOD reagent buffer were diluted in 1 L of distilled water to prepare solution 1. Then, the contents of one bottle of GOPOD reagent enzymes were dissolved in approximately 20 mL of solution 1 and transferred to a labelled bottle. The remaining solution 1 was added to the bottle, which was then covered with aluminium foil to protect the reagent from light. This solution is the glucose determination reagent (GOPOD reagent), which is stable at 4°C for 1 to 4 months. For storage beyond 4 months, the reagent should be aliquoted and stored at -10°C.

3.5.1.3.2. Sample preparation and kinetics of starch digestion

The biscuits were ground using a grinder to achieve a fine powder. To ensure a uniform sample size for *in vitro* GI analysis, the powdered sample was sieved through a 1-mm mesh sieve. A portion of the sample, containing 50 ± 5 mg of powdered biscuit, was prepared in triplicate tubes. To each tube, 10 mL of HCl-KCl buffer (0.2M, pH 1.5) and 0.2 mL of pepsin solution were added, and the tubes were incubated at 40°C with shaking at 300 rpm for 1h to simulate the *in vivo* gastric phase and prevent protein-starch interactions. After incubation, 25 mL of Tris-maleate buffer solution (0.1M, pH 6.9) was added to the mixture to inactivate the pepsin reaction and establish optimal conditions for the small intestinal phase. The small intestinal phase was then simulated by adding 5 mL of α-amylase solution, prepared as described in the previous section and stored on ice, to mimic human saliva. The mixture was then incubated at 37°C with shaking at 300 rpm. Then, 1 mL of aliquots of the sample solutions was taken out and placed in flasks at different time points (t =20, 30, 60, 90, 120, 150, and 180 min). Subsequently, the aliquots were placed in a water bath at 100°C for 5 min to stop the enzymatic activity. The flasks were then stored on ice until the completion of digestion. Once all samples were collected, the flasks were stored at 4°C until further analysis the following day.

The following day, all flasks were taken out of the storage at 4°C. The next step involved hydrolysing the digested starch into glucose by adding 3 mL of sodium acetate buffer (0.4M, pH 4.75) and 60 µL of amyloglucosidase. The flasks were then incubated at 60°C for 45 min, with shaking at 300 rpm. After incubation, the flasks were allowed to cool to ambient temperature. The process was then continued with the GOPOD procedure using the GOPOD D-glucose assay kit (Megazyme, Ireland). A 0.1 mL aliquot from each sample was transferred into clearly labelled test tubes in triplicate. Reagent blanks were prepared by adding 0.1 mL of distilled water to the bottom of two test tubes. The D-glucose standards were made by adding 0.1 mL of the D-glucose standard solution (provided in the K-GLUC kit) to the bottom of test tubes in quadruplicate. Subsequently, 3.0 mL of GOPOD Reagent, prepared as described in the previous section, was added to each aliquot, blank solution, and D-glucose standard. All test tubes were incubated at 45°C for 20 min. The absorbance of each sample was measured at 510 nm against the reagent blank using a spectrophotometer (UVmini-1240, SHIMADZU, Japan).

3.5.1.3.3. Estimated glycaemic index (eGI) of biscuits

The concentration of D-glucose in the sample ($C_{glucose}$), expressed in g/L, was calculated using the "Megazyme Mega-Calc™ Data Calculator**".** The glucose concentration was then converted to starch content by multiplying by 0.9 (Goni & Saura-Calixto, 1997). Therefore, the partially hydrolysed starch in the sample ($St_{pr,1}$ in g/L or mg/mL) is:

$$St_{pr,1} = C_{glucose} \cdot 0.9 \qquad \textit{Eq. 3.19}$$

The partially hydrolysed starch (St_{pr}) in mg is calculated as follows:

$$St_{pr} = St_{pr,1} \cdot 4 \cdot 30 \qquad \textit{Eq. 3.20}$$

The TS in the sample (TS_s), expressed in mg, was calculated using the TS, TS per 100 g, of the sample, reported by the laboratory, and the mass of the sample (m):

$$TS_s = TS \cdot m \cdot 10 \qquad \textit{Eq. 3.21}$$

The percentage of partially hydrolysed starch (% St_{pr}) is:

$$\%\, St_{pr} = \frac{St_{pr}}{TS_s} \cdot 100 \qquad \textit{Eq. 3.22}$$

The eGI of the biscuits, with white bread as the reference, ($eGI_{s,br}$) was calculated using the equation below:

$$eGI_{s,br} = 39.21 + 0.803 \cdot \%\, St_{pr} \qquad \textit{Eq. 3.23}$$

The $eGI_{s,br}$ was converted to the eGI with glucose as the reference ($eGI_{s,gl}$) using a factor of 0.73.

$$GI_{s,gl} = GI_{s,bread} \cdot 0.73 \qquad \textit{Eq. 3.24}$$

3.6. Fibre-added laminated biscuits

Fibre-added laminated biscuits were developed in collaboration with the International Hellenic University (IHU) in Thessaloniki, Greece, as part of a three-month international mobility project. In this collaboration, a portion of the wheat flour in laminated biscuits was replaced with raw carob (LB-CA) or acorn flour (LB-AC) to improve the nutritional quality of the final product. Initial recipe adjustments were made in Greece using a small-scale mixer and oven. Subsequently, production was scaled up in Nantes at the GEPEA laboratory using a VMI prototype mixer. This project was carried out under the supervision of Prof. Maria Papageorgiou and in collaboration with Dr. Anna Marinopoulou.

3.6.1. Primary ingredients for fibre-added biscuit

Fibre-added biscuit doughs were prepared using type T55 wheat flour (Enilia, France), characterised by a water content of 14.34% (w.b.), protein content of 9.60%, damaged starch level of 14.3 UCD, and 0.58% ash. This flour was a biscuit flour made from soft wheat. Raw carob Bio flour was supplied by Creta Carob (Greece) (Figure 3.21), and acorn flour was provided by Red Tractor Farm of Kea Island (Greece) (Figure 3.22). Additional ingredients included 82% fat butter (Président, France), icing sugar (Béghin Say, France), salt, water, glucose syrup (Vahiné, France), ABC and SBC (both from Brenntag, Germany), and SAPP 10 (Budenheim, Germany). SAPP 10 was chosen as a slow-acting LA, releasing only 10% of the CO_2 from SBC during dough mixing.



Nutrient	Amount
Energy	318 kcal / 1331 kJ
Fats	0.4 g
Saturated Fats	0 g
Carbohydrates	58.9 g
Sugars	46.99 g
Dietary Fibers	30 g
Proteins	4.7 g
Salt	0 g

Figure 3.21. Raw carob flour (Creta, Greece) and its nutrition facts per 100 g.



Nutrient	Amount
Fats	8.1 g
Carbohydrates	66.5 g
Dietary Fibers	15.2 g
Proteins	4.6 g
Ash	0.5 g

Figure 3.22. Acorn flour and its nutrition facts per 100 g.

3.6.2. Physical and functional properties of acorn and carob flours

3.6.2.1. Water content

The method described in Section 3.2.1.2.2 was used to measure the water content of the flours.

3.6.2.2. Colour

Flours' colour was measured using a MetaVue colourimeter (X-rite, US), following the CIELAB colour space system. This system quantifies colour using three parameters: L* (lightness), ranging from 0 (black) to 100 (white); a*, indicating the red–green axis (negative values denote green, positive values indicate red); and b*, representing the blue–yellow axis (negative values for blue, positive values for yellow). On each flour, colour readings were taken at three distinct surface points, and the mean value was calculated. The instrument was routinely calibrated to ensure measurement accuracy throughout the analysis.

3.6.2.3. Bulk density

The bulk density of flours was measured following the method outlined by Elkhalifa et al. (2005). A 25 ml graduated cylinder was filled with 10 g of the fibre sample. To give the fibre time to settle, the cylinder was gently tapped ten times. Following tapping, the final volume was measured, and the bulk density was determined and expressed as grams per millilitre (g/mL).

3.6.2.4. Water holding capacity (WHC)

1 g of each fibre sample was placed into a 50 mL centrifuge tube. 40 mL of distilled water was then added. The mixture was stirred thoroughly and left to stand at room temperature for one hour. After this period, the samples were centrifuged at 10000 RCF for 15 min. The supernatant was then discarded, and the remaining pellet was weighed. The water holding capacity (WHC) was expressed as grams of retained water per gram of fibre sample (Rana et al., 2015).

3.6.2.5. Oil holding capacity (OHC)

20 mL of sunflower oil was added to pre-weighed centrifuge tubes containing 2 g of fibre. The mixture was thoroughly mixed using a vortex, then left undisturbed at 25 ± 2°C for 30 min. Afterward, the tubes were centrifuged at 10000 RCF for 15 min, and the excess oil was carefully decanted. Oil holding capacity (OHC) was determined and expressed as grams of oil retained per gram of fibre sample (Elkhalifa et al., 2005).

3.6.2.6. Swelling capacity (SWC)

1 g of each fibre sample was placed into a graduated cylinder, and 30 mL of water was added. The samples were then left to hydrate for 18 h. After hydration, the final volume occupied by the fibre samples was measured, and the swelling capacity (SWC) was expressed as the occupied volume in mL per gram of fibre sample (Robertson et al., 2000).

3.6.3. Formulation

Multiple recipes were trialled to ensure compatibility with the forming machines and to achieve the desired final product density. Five types of biscuits were produced:

Laminated biscuits containing LAs (LB-ABC): The dough was mixed at atmospheric pressure. The LB-ABC biscuit is the control.

Laminated biscuits containing LAs, with 20% wheat flour replaced with raw carob flour (LB-CA-20): 20% of the wheat flour was replaced with raw carob flour, and the dough was mixed at atmospheric pressure.

Laminated biscuits containing LAs, with 20% wheat flour replaced with acorn flour (LB-AC-20): 20% of the wheat flour was replaced with acorn flour, and the dough was mixed at atmospheric pressure.

Laminated biscuits containing LAs, with 30% wheat flour replaced with raw carob flour (LB-CA-30): 30% of the wheat flour was replaced with raw carob flour, and the dough was mixed at atmospheric pressure.

Laminated biscuits containing LAs, with 30% wheat flour replaced with acorn flour (LB-AC-30): 30% of the wheat flour was replaced with acorn flour, and the dough was mixed at atmospheric pressure.

The composition of these biscuits is expressed per 100 g (w.b.) of flour (Table 3.6).

Table 3.6. Composition of fibre-added laminated biscuits per 100 g of flour.

	Mass of ingredients (g) per 100 g of flour (w.b)				
Ingredients	LB-ABC	LB-CA-20	LB-CA-30	LB-AC-20	LB-AC-30
Flour	100	80	70	80	70
Raw carob flour	-	20	30	-	-
Acorn flour	-	-	-	20	30
Butter	5.53	5.53	5.53	5.53	5.53
Icing sugar	31.85	31.85	31.85	31.85	31.85
Water	20.77	22.00	22.66	20.77	20.77
ABC	1.00	1.00	1.00	1.00	1.00
SBC	0.64	0.64	0.64	0.64	0.64
SAPP 10	0.40	0.40	0.40	0.40	0.40
Salt	0.94	0.94	0.94	0.94	0.94
Glucose syrup	2.23	2.23	2.23	2.23	2.23
Total	**163.36**	**164.59**	**165.25**	**163.36**	**163.36**
Dough hydration (%)	**22.44**	**22.46**	**22.41**	**22.22**	**22.11**

3.6.4. Mixing procedure and the dough texture

The fibre-added biscuit dough was prepared using the all-in method, similar to laminated biscuit dough. In this method, all ingredients are added to the mixer before the mixing process begins. Prior to mixing, glucose syrup was diluted in a small amount of water, and ABC was dissolved in another portion of water. The remaining water was combined with icing sugar and salt and thoroughly mixed to avoid clumping. All ingredients were then added at once into the mixer, which was equipped with a single spiral blade. The mixing process was carried out using a prototype batch mixer (VMI, St-Hilaire-de-Loulay, France). For each test, a 3 kg batch of dough was prepared. The biscuit dough mixing procedure is illustrated in Figure 3.23.

<table>
<tr><th>LBCR20</th><th>LBCR30</th><th>LBAC20</th><th>LBAC30</th></tr>
<tr><td>Solutions:
Icing sugar + Salt + Water
ABC + Water
Glucose syrup + Water</td><td>Solutions:
Icing sugar + Salt + Water
ABC + Water
Glucose syrup + Water</td><td>Solutions:
Icing sugar + Salt + Water
ABC + Water
Glucose syrup + Water</td><td>Solutions:
Icing sugar + Salt + Water
ABC + Water
Glucose syrup + Water</td></tr>
<tr><td colspan="4">For each type of dough, the prepared solutions are combined to form a single solution, referred to as “Solution A”</td></tr>
<tr><td>Flour (wheat + carob)
SBC + SAPP10 +
Butter + Solution A
(all in the mixer)</td><td>Flour (wheat + carob)
SBC + SAPP10 +
Butter + Solution A
(all in the mixer)</td><td>Flour (wheat + acorn)
SBC + SAPP10 +
Butter + Solution A
(all in the mixer)</td><td>Flour (wheat + acorn)
SBC + SAPP10 +
Butter + Solution A
(all in the mixer)</td></tr>
<tr><td colspan="4">1 min air flushing at -0.9 bar (Tool: 50 rpm; satellite: 5 rpm) with partially open valve
1 min air injection (Tool: 100 rpm; satellite: 5 rpm) at atmospheric pressure with partially open valve
8 min air injection (Tool: 100 rpm; satellite: 10 rpm) at atmospheric pressure with partially open valve
Temperature: 30°C</td></tr>
<tr><td colspan="4">30 min dough rest at 27°C</td></tr>
</table>

Figure 3.23. Fibre-added laminated biscuits mixing procedure.

Texture profile analysis (TPA) was performed for biscuit dough made from wheat flour and the proposed combinations of wheat flour with acorn and raw carob flours, after dough rest at 27°C for 30 min, using a texturometer TA.XT.plus (Stable Micro Systems, England) equipped with a load cell of 5 kg. The rheological characteristics were measured following the method applied by Maache-Rezzoug et al. (1998b). Dough samples were formed by placing them in a cylindrical container with 30 mm in diameter and a height of 17 mm. The sample was compressed between parallel plates, lubricated with oil to eliminate friction between the sample and the plates. A single compression at the speed of 0.7 mm/s was applied. Samples were compressed to 50% of their initial height.

3.6.5. Forming procedure

After resting for 30 min at 27°C, the fibre-enriched biscuit dough was sheeted using a RONDO dough sheeter (RONDO, Germany). Lamination was performed in five stages, with the roll nips set at 10, 8, 6, 4, and 2 mm. Once laminated, the dough was shaped manually with a 60 × 60 mm rectangular cutter fitted with needles. Forming occurred 1 min after lamination to reduce shrinkage. The biscuits were then placed on an oven rack for baking, with 28 biscuits baked per batch.

3.6.6. Baking procedure

The biscuits were baked in a deck oven (MIWE, Germany), with the top temperature set to 240°C and the bottom temperature to 250°C. Biscuits containing carob flour (LB-CR-20 and LB-CR-30) were baked for 8 min and 30 s, while those with acorn flour (LB-AC-20 and LB-AC-30) were baked for 9 min. During

the first 2 min of baking, 140 mL of saturated steam was injected into the oven to simulate industrial baking conditions.

For each batch, 28 biscuits were placed on the oven tray, and 8 biscuits were selected and marked for further analysis. After baking, the biscuits were allowed to cool at room temperature for 30 min before being sealed in a plastic bag for further analysis.

3.6.7. Characterisation methods

The methods described in Section 3.2.1.2 of the Materials and Methods were used for the characterisation of fibre-added biscuits, except for the fibre content.

The total dietary Fibre (TDF) content of the biscuits was measured by an external laboratory using an enzymatic-gravimetric method.

3.6.8. Sensory analysis

Fibre-added biscuits were prepared a week in advance. They were stored in sealed bags at ambient temperature in the laboratory. The panel to study the influence of added fibre on overall biscuit preference evaluated five different biscuits and distinct biscuit features: LB-ABC-CB as a control, LB-CA-20, LB-AC-20, LB-CA-30, and LB-AC-30.

The general conditions were consistent with those described in Section 3.4.4.1 of the Materials and Methods. A panel of 85 judges conducted a hedonic test in combination with a JAR test in a single session. The composition of the panel is shown in Figure 3.24.

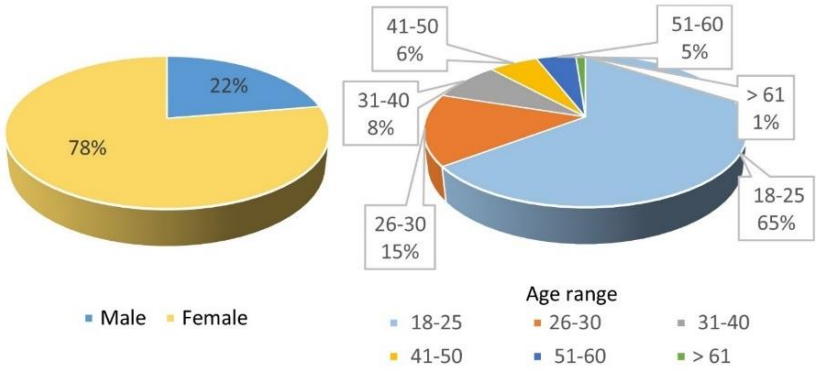

Figure 3.24. Composition of the panel used for the hedonic and JAR tests on fibre-added biscuits (n = 85).

3.6.8.1.1. Hedonic test

This test was conducted similarly to the hedonic test applied to laminated and rotary biscuits, as described earlier (Section 3.4.4.2.1).

3.6.8.1.2. Just about right (JAR) test

This test aimed to improve consumer satisfaction by assessing the degree of satisfaction with several product attributes as follows:

- Colour
- Hardness
- Friability
- Graininess
- Sweetness
- Bitterness
- Overall aromatic intensity
- Cacao aroma

A 5-point scale was proposed, ranging from 1 (really not enough) to 5 (really too much). The JAR test rating scale is thus double-opposed. The median response (3) represents the “just right” level for the characteristic according to the judge, while the extremes are two contradictory levels of non-satisfaction.

3.7. Simplified mass-energy balance approach for biscuit baking

This part of the Materials and Methods considers the energy required for baking biscuits, specifically the isosteric heat of desorption needed to remove water in the low water content foods, like biscuits. This approach is developed theoretically and was conceptualised with the assistance of Prof. Alain Le-Bail. The objective of this part is to estimate the total energy required to bake dough pieces from their initial state to a final product with a target water content (~4% w.b.). A simplified approach based on coupled mass and energy balances is proposed, using clearly stated assumptions. The approach evaluates the total energy required between the initial and final states, including the energy associated with water evaporation and desorption.

In order to calculate the baking energy, it was hypothesised that water evaporation in dough pieces during baking occurs in two steps: first, it was assumed that 50% of the water, the non-bound (free) water, evaporates at approximately 105°C. This temperature was chosen because the boiling point of a water-sugar solution increases with the concentration of sugar. The more sugar dissolved, the higher the boiling point. A saturated sugar solution, similar to ours, has a boiling point around 105°C. In the second step, the remaining water, which is bound water, evaporates until reaching the final water content in baked biscuits (~ 4% w.b.). The calculations are as follows:

The specific heat of the dough ($C_{p,dough}$) was calculated using the mass fraction (X_i) and the specific heat (C_i) of each ingredient, according to the following equation:

$$C_{p,dough} = \sum_i X_i \cdot C_i \qquad \textit{Eq. 3.25}$$

Assuming the water content of the baked biscuit on a wet basis is X_{W-WB}, the water content of the final product on a dry basis (X_{W-DB}) was calculated as follows:

$$X_{W-WB} = \frac{m_{W-dough}}{m_{W-dough} + m_{DM-dough}} \qquad \textit{Eq. 3.26}$$

$$X_{W-DB} = \frac{m_{W-dough}}{m_{DM-dough}} \qquad \textit{Eq. 3.27}$$

where $m_{W-dough}$ and $m_{DM-dough}$ are the mass of water and the mass of dry matter in the dough, respectively. Therefore,

$$m_{W-dough} = X_{W-WB} \cdot m_{W-dough} + X_{W-WB} \cdot m_{DM-dough} \quad \textit{Eq. 3.28}$$

$$m_{W-dough} \cdot (1 - X_{W-WB}) = X_{W-WB} \cdot m_{DM-dough} \quad \textit{Eq. 3.29}$$

$$X_{W-DB} = \frac{m_{W-dough}}{m_{DM-dough}} = \frac{X_{W-WB}}{1 - X_{W-WB}} \quad \textit{Eq. 3.30}$$

Assuming the final water content of 4% in baked biscuits ($X_{W-WB} = 0.04$), the X_{W-DB} is calculated as follows:

$$X_{W-DB} = \frac{X_{W-WB}}{1 - X_{W-WB}} = \frac{0.04}{1 - 0.04} = 0.0323 \quad \textit{Eq. 3.31}$$

where $m_{W-dough}$ and $m_{DM-dough}$ were calculated using the mass (m_{i-WB}) and the water content (X^i_{W-WB}) of each ingredient on a wet basis:

$$m_{W-dough} = \sum_i m_{i-WB} \cdot X^i_{W-WB} \quad \textit{Eq. 3.32}$$

$$m_{DM-dough} = (\sum_i m_{i-WB}) - m_{W-dough} \quad \textit{Eq. 3.33}$$

The initial mass of the water in the biscuit (or the dough piece) (m^0_{W-Bis}) and the initial mass of the biscuit (m^0_{Bis}) are:

$$m^0_{W-Bis} = m_{W-dough} \quad \textit{Eq. 3.34}$$

$$m^0_{Bis} = m^0_{W-Bis} + m_{DM-dough} \quad \textit{Eq. 3.35}$$

As mentioned earlier, it was assumed that 50% of the water in the biscuit dough evaporated during the first step, where the biscuits are semi-baked. Therefore, the mass of the water in the biscuit (m^{step1}_{W-Bis}) and the total mass of the biscuit at the end of the first step (m^{step1}_{Bis}) are:

$$m^{step1}_{W-Bis} = 0.5 \cdot m_{W-dough} \quad \textit{Eq. 3.36}$$

$$m^{step1}_{Bis} = m^{step1}_{W-Bis} + m_{DM-dough} \quad \textit{Eq. 3.37}$$

Therefore, the mass of the semi-baked biscuit per 1 g of initial dough ($m_{Bis,semi-baked}$) is:

$$m_{Bis,semi-baked} = \frac{m_{Bis}^{step1}}{\sum_i m_{i-WB}} \quad \textit{Eq. 3.38}$$

In the second step, the remaining water is evaporated to achieve the target water content in the baked biscuits (X_{W-WB}). The mass of the baked biscuit (m_{Bis}^{step2}) at this stage was determined using the $m_{DM-dough}$ and the mass of the water in the biscuit at the end of the second step (m_{W-Bis}^{step2}), along with the X_{W-WB} :

$$m_{W-Bis}^{step2} = \frac{X_{W-WB}}{1 - X_{W-WB}} \cdot m_{DM-dough} \quad \textit{Eq. 3.39}$$

$$m_{Bis}^{step2} = m_{W-Bis}^{step2} + m_{DM-dough} \quad \textit{Eq. 3.40}$$

The mass of the baked biscuit per 1 g of initial dough ($m_{Bis,baked}$) is:

$$m_{Bis,baked} = \frac{m_{Bis}^{step2}}{\sum_i m_{i-WB}} \quad \textit{Eq. 3.41}$$

The water loss ratios between the different steps of baking on a dry basis can be calculated as follows:

$$Water\ loss_{s1-so}^{DB}\ (\%) = \frac{m_{W-Bis}^{step1} - m_{W-dough}}{m_{DM-dough}} \quad \textit{Eq. 3.42}$$

$$Water\ loss_{s2-s1}^{DB}\ (\%) = \frac{m_{W-Bis}^{step2} - m_{W-Bis}^{step1}}{m_{DM-dough}} \quad \textit{Eq. 3.43}$$

$$Water\ loss_{s2-s0}^{DB}\ (\%) = \frac{m_{W-Bis}^{step2} - m_{W-dough}}{m_{DM-dough}} \quad \textit{Eq. 3.44}$$

$$Water\ loss_{s2-s0}^{DB}\ (\%) = \frac{m_{W-Bis}^{step2} - m_{W-dough}}{m_{DM-dough}} \quad \textit{Eq. 3.45}$$

where $Water\ loss_{s1-so}^{DB}$, $Water\ loss_{s2-s1}^{DB}$, and $Water\ loss_{s2-s0}^{DB}$ represent the water loss between the first and initial steps, the second and first steps, and the second and initial steps, respectively.

Assuming an initial baking temperature of 30°C (T_0), the energy required to heat the dough (Q_{sens1}), in J, to 105°C (T_1) is calculated using the equation below:

$$Q_{sens1} = m_{Bis}^{0} \cdot C_{p,dough} \cdot \Delta T \quad \textit{Eq. 3.46}$$

The energy required for evaporating water in the dough (Q_{vap}) is calculated with the mass of semi-baked biscuit ($m_{Bis,semi-baked}$) and the latent heat of water evaporation (L_v) as follows:

$$Q_{vap} = \left(m_{Bis}^{0} - m_{Bis,semi-baked}\right) \cdot L_v \qquad \textit{Eq. 3.47}$$

Therefore, the total energy required to evaporate 50% of the water (Q_1) in the dough is:

$$Q_1 = Q_{sens1} + Q_{vap} \qquad \textit{Eq. 3.48}$$

Considering that the temperature of the baked biscuits reaches 140°C at the end of the baking process to achieve the target water content of 4% (w.b.), the energy required to convert the semi-baked biscuit into the baked biscuit (Q_2) was calculated. This energy includes the heat needed to raise the temperature of the dough (Q_{sens2}) from 105°C (T_1) to 140°C (T_2), as well as the "desorption energy" (Q_{ist}). Desorption is the process by which a substance (a gas or liquid) is released from or separated from a surface or material to which it was previously adsorbed. The desorption energy is the heat required to release the adsorbed substance. The "isosteric heat of desorption" (L_{ist}) is the heat associated with the desorption process under conditions where the number of adsorbed molecules remains constant.

$$Q_{sens2} = m_{Bis,semi-baked} \cdot C_{p,semi-Bis} \cdot \Delta T \qquad \textit{Eq. 3.49}$$

where the specific heat of the dough after evaporation of half of the water ($C_{p,semi-Bis}$) was determined using the $C_{p,dough}$, m_{W-Bis}^{step1}, and the specific heat of water ($C_{p-water}$), as described by the following equation:

$$C_{p,semi-Bis} = C_{p,dough} - \left(m_{W-Bis}^{step1} \cdot C_{p-water}\right) \qquad \textit{Eq. 3.50}$$

Thus,

$$Q_{ist} = \left(m_{Bis,baked} - m_{Bis,semi-baked}\right) \cdot L_{ist} \qquad \textit{Eq. 3.51}$$

$$Q_2 = Q_{sens2} + Q_{ist} \qquad \textit{Eq. 3.52}$$

The total energy required for baking the biscuit ($Q_{total-Bis}$) is:

$$Q_{total-Bis} = Q_1 + Q_2 \qquad \textit{Eq. 3.53}$$

The steps of biscuit baking are summarised in Figure 3.25.

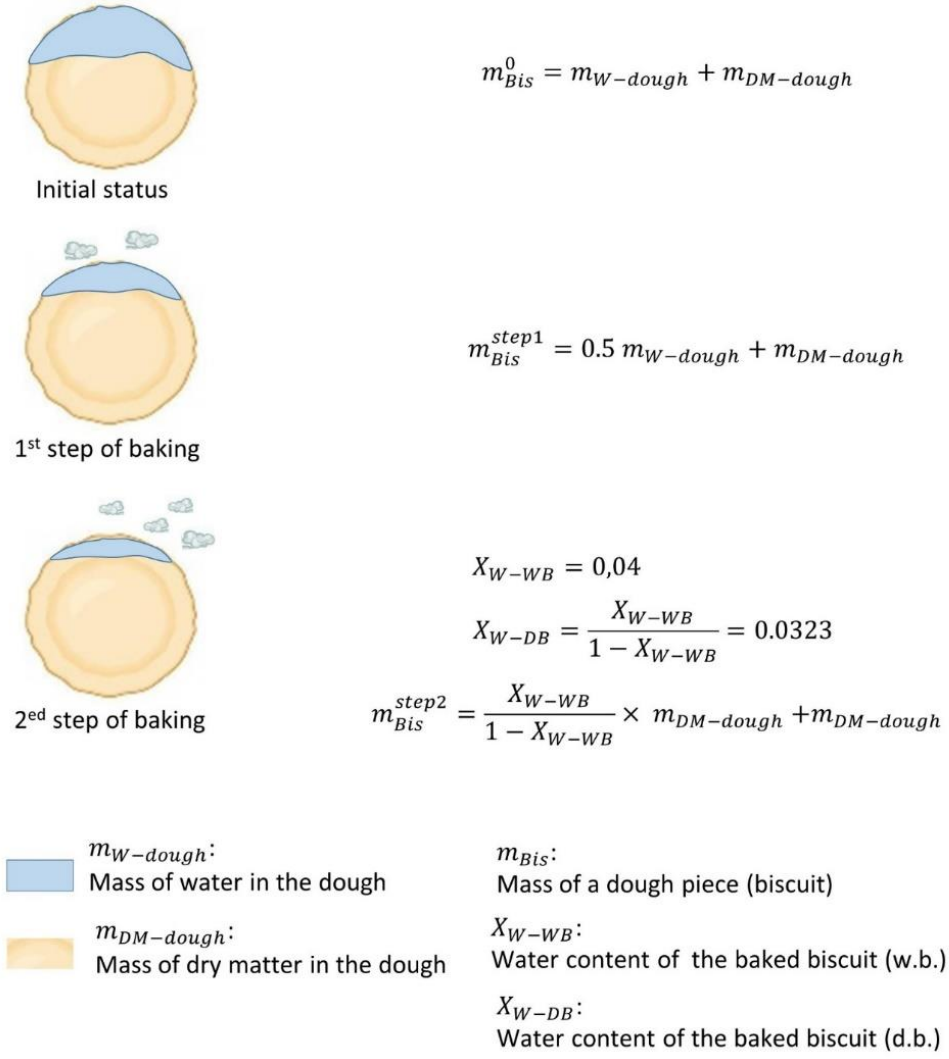

Figure 3.25. Steps of biscuit baking in an oven; the biscuit baking process involves two steps: it is assumed that 50% of the water is evaporated during the first step, and the remaining water is removed in the second step to achieve the target final water content of 4% (w.b.).

3.8. Statistical analysis

Data validation was performed using one-way analysis of variance (ANOVA) in STATGRAPHICS 19 software (Statgraphics Technologies, US). Significant difference analysis was carried out at the 95% confidence interval. ANOVA was followed by Fisher's Least Significant Difference (LSD) post hoc test.

Principal Component Analysis (PCA) was used to simplify the data and find patterns while preserving a significant amount of information as multiple variables were being examined. Additionally, it facilitates the discovery of variable correlations. XLSTAT (2022.3.1.1333, Addinsoft, France) was used to perform PCA.

The results of the CATA and JAR tests were analysed using XLSTAT (2022.3.1.1333, Addinsoft, France).

4. RESULTS AND DISCUSSION

4.1. Selection of flour, optimal mixing, and baking conditions

The quality of biscuits is greatly influenced by the choice of flour, mixing, and baking conditions. In order to produce biscuits with the desired texture, structure, and appearance, this part of the Results and Discussion sought to optimise the type of flour, dough mixing parameters, and baking conditions. Furthermore, the effect of CO_2 over-pressure mixing on biscuit quality and dough aeration was assessed. To gain a better understanding of its role in dough porosity, the solubility behaviour of CO_2 under various mixing protocols was also examined.

4.1.1. Selection of flour

Since the role of flour in biscuit manufacturing is important, three types of flours, T65 (Evelia), T55 (Paulic), and T55 (Enilia), were assessed to choose the appropriate one for making laminated and rotary biscuits. The main characteristics of these flours are summarised in Table 4.1. Some characteristics of Evelia (France) flour, including protein content and P/L, were provided by the supplier, while the remaining measurements were made in the GEPEA laboratory. The characteristics of Paulic (France) and Enilia (France) flours were analysed by an external laboratory.

Table 4.1. Characteristics of Evelia, Paulic, and Enilia flours.

Type of flour	Alveograph Parameters				Protein (%)	Damaged starch (UCD)
	P (mm)	L (mm)	W (10^{-4} J)	P/L		
T65 (Evelia)	94	102	366	0.88	9.5	-
T55 (Paulic)	60	80	152	0.75	9.4	23.4
T55 (Enilia)	24	100	66	0.24	9.6	14.3

Unlike bread, the formation of a gluten network in biscuits is not desirable. The choice of flour and the type of mixture actually limit its development. In biscuit making, low-strength flours are chosen (W < 150), as the dough must be stretchy and non-elastic during the manufacturing process. Biscuit flour also has a lower P/L ratio than bread flour, in the range of 0.3–0.5. Flours for biscuits with a low protein content and low damaged starch content are preferred (Misra & Tiwari, 2014). This is because a high proportion of damaged starch granules tends to increase the water absorption of the flour. Since the biscuit baking process is essentially a drying process, biscuit formulation aims to add as little water as possible, as this directly affects the dough's hydration level. Similarly, the higher the protein content, the greater the amount of water absorbed by the proteins, allowing gluten to form. The dough is then

less hydrated and more crumbly, and the biscuit has a more compact texture after baking (Maache-Rezzoug et al., 1998a). A very limited gluten network is formed in the case of laminated biscuits. However, this network may contribute to form change during baking and to cracking during storage, as proteins allow gluten to form. Therefore, a low gluten content is expected. According to the observed characteristics, Enilia flour demonstrated better properties; however, the density of biscuits made with the selected flours was also studied. All biscuits were baked at 250°C with 180 mL water injected in the form of steam in the first phase of baking, and the total duration of 8 min in a conventional oven (MIWE, Germany). The characteristics of the laminated biscuits made with these three flours are shown in the table below (Table 4.2):

Table 4.2. Characteristics of laminated biscuits made with Evelia, Paulic, and Enilia flours.

	Biscuit dimensions			
Type of flour	Length (mm)	Width (mm)	Thickness (mm)	Density (g/ cm^3)
Evelia	62.45 ± 0.97	50.80 ± 1.16	8.30 ± 0.46	0.45 ± 0.01
Paulic	63.13 ± 1.65	52.35 ± 1.04	11.18 ± 0.14	0.43 ± 0.01
Enilia	64.27 ± 0.98	566.80 ± 1.85	11.89 ± 0.31	0.28 ± 0.02

As mentioned earlier, a density below 0.3 g/cm^3 for the final baked biscuit, defined by the biscuit industry in France (ARVALIS et al., 2022), is expected to qualify a biscuit flour.

4.1.2. Optimal mixing conditions for laminated biscuit dough containing leavening agents (LAs)

To find the optimal mixing conditions for laminated biscuit dough containing LAs, the mixing was performed in three different rotation speeds in a prototype VMI mixer (St-Hilaire-de-Loulay, France) with a spiral blade, and the void fraction of dough was measured (Table 4.3).

Table 4.3. Void fraction of laminated biscuit dough containing LAs for different mixing conditions.

Experiment	Stage 1: Pre-mixing (min)	Stage 2: Mixing (min)	Stage 3: Kneading (min)	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage	Void fraction (%)
E1	1	2	5	30	50,100,150	5,5,10	14.49 ± 0.95 [a]
E2	1	2	8	30	50,100,150	5,5,10	13.36 ± 0.89 [b]
E3	1	2	10	30	50,100,150	5,5,10	13.39 ± 0.32 [b]
E4	1	2	15	30	50,100,150	5,5,10	12.41 ± 0.48 [c]
E5	1	1	8	30	50,100,100	5,5,10	12.92 ± 0.43 [b]

Dough kneading at the speed of 150 rpm for 5 min resulted in the highest dough void fraction (14.49 ± 0.95). The dough void fraction at the kneading speed of 150 rpm for 8 min and 10 min were both placed in the second place. The dough void fraction at the kneading speed of 100 rpm for 8 min was placed after; however, no significant difference was observed between this kneading condition and those placed in the second place. The findings show that the void fraction of dough decreased with increasing kneading time at the same speed. The void fraction shows how much of the dough is made up of air and gas bubbles. The void fraction in the biscuits is an important factor that governs the mechanical and fracture properties of the biscuits, which are important in biscuit processing, packaging, and structure development (Baltsavias et al., 1999). As one can expect, there is a direct relation between the void fraction and the biscuit's porosity after baking. A higher void fraction is therefore expected to improve the texture of the final product. Although kneading at the speed of 150 rpm for 5 min had the highest void fraction, the mixing was not complete, and particles of flour were observed in the dough after mixing. As there was not a significant difference between the 8 min kneading at the speed of 150 and 100 rpm, the kneading at the speed of 100 rpm was selected as the optimal condition in order to reduce the energy consumption. This mixing duration also aligns with the mixing times commonly observed in the industry.

4.1.3. Optimal mixing conditions for CO_2 over-pressure mixing of leavening agents-free (LAs-free) laminated biscuit dough

To define the optimal mixing conditions for biscuit dough free from LAs using CO_2 over-pressure mixing, three different kneading speeds were applied for a constant kneading time of 8 min at 30°C, and the dough void fraction was measured (Table 4.4).

Table 4.4. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at different kneading speeds.

Experiment	Stage 1: Pre-mixing (min)	Stage 2: Mixing (min)	Stage 3: Kneading (min)	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage	Void fraction (%)
E1	1	2	8	30	50,100,100	5,5,10	13.29 ± 0.80 [c]
E2	1	2	8	30	50,100,120	5,5,10	17.29 ± 1.17 [b]
E3	1	2	8	30	50,100,180	5,5,10	18.32 ± 1.21 [a]

ANOVA revealed a significant difference in dough void fraction of different kneading speeds. The kneading speed of 150 rpm was selected as showed a higher void fraction. This finding aligns with the finding of Chin and Campbell (2005), who indicated that dough void fraction increases with mixing speed.

In the second step, the impact of kneading time was assessed (Table 4.5).

Table 4.5. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at different kneading times.

Experiment	Stage 1: Pre-mixing (min)	Stage 2: Mixing (min)	Stage 3: Kneading (min)	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage	Void fraction (%)
E4	1	2	5	30	50,100,180	5,5,10	16.22 ± 0.48 [b]
E5	1	2	10	30	50,100,180	5,5,10	18.32 ± 1.21 [a]
E6	1	2	15	30	50,100,180	5,5,10	14.88 ± 0.87 [c]

Findings revealed that kneading for 10 min gave the highest void fraction (18.32% ± 1.21). By increasing the kneading time from 10 min to 15 min, the void fraction reduced to 14.88% ± 0.87, which could be attributed to degassing of the dough caused by the breakage of gas bubbles due to increased kneading time.

In the next step, the void fraction of the dough was studied at a temperature of 25°C to see how mixing temperature affected the dough void fraction (Table 4.6). The temperature was lowered to 25°C to

enhance the solubility of CO_2 in the dough; indeed, CO_2 solubility in water is known to increase as temperature decreases.

Table 4.6. LAs-free laminated biscuit dough void fraction under CO_2 over-pressure mixing at 30°C and 25°C.

Experiment	Stage 1: Pre-mixing (min)	Stage 2: Mixing (min)	Stage 3: Kneading (min)	Temperature (°C)	Agitator speed (rpm) in each stage	Satellite speed (rpm) in each stage	Void fraction (%)
E7	1	2	10	30	50,100,180	5,5,10	18.32 ± 1.21
E8	1	2	10	25	50,100,180	5,5,10	20.11 ± 0.65

The void fraction of the laminated dough at 25°C was higher (20.11% ± 0.65). This could be justified by the higher solubility of CO_2 at lower temperatures and by the fact that CO_2 is more soluble in water and lipids than in air. This finding is in accordance with the findings of Jakobson et al. (Jakobsen et al., 2009). They studied the solubility of CO_2 in semi-hard cheeses. They found that CO_2 is highly soluble in both the water phase and the fat phase. As temperature increases, CO_2 solubility in the water phase decreases, while it increases in the fat phase.

Therefore, 10 min kneading at the speed of 180 rpm, and pre-mixing and mixing for 1 min and 2 min, respectively, at the temperature of 25°C, was selected as the optimal mixing condition for the LAs-free laminated biscuit dough under CO_2 over-pressure mixing.

4.1.4. Optimal baking conditions for laminated biscuits

To determine the optimal baking conditions, biscuits were baked at various temperatures, baking times, and quantities of water injection in the form of steam. The HygroxTm-P4 McQueen (England) was placed in the oven to monitor the specific humidity and temperature in the oven during baking. Biscuit attributes, including density, dimensions, and the water content, were examined to determine the most suitable baking conditions (Table 4.7).

Table 4.7. Laminated biscuit (containing LAs) attributes at various baking conditions.

Baking temperature (°C)	Bking duration	Injected water in form of steam (mL)	Lenght of biscuit (mm)	Width of biscuit (mm)	Thickness of biscuit (mm)	Density (g/cm^3)	Water content % (d.b)
260	5 min 30 s	140	63.29 ± 0.91	56.93 ±0.79	12.14 ± 0.53	0.30 ± 0.1	7.63 ± 0.16
260	5 min 30 s	280	64.40 ± 1.07	57.13 ± 0.86	11.99 ± 0.33	0.29 ± 0.1	7.87 ± 0.17
240	9 min	140	63.09 ± 1.15	59.80 ± 1.02	13.51 ± 0.48	0.24 ± 0.1	4.03 ± 0.48
240	9 min	180	64.90 ± 1.05	60.46 ± 1.29	12.81 ± 0.29	0.23 ± 0.1	3.62 ± 0.64
240	9 min 30 s	140	63.96 ± 0.32	59.55 ± 0.85	9.71 ± 0.23	0.33 ± 0.1	3.51 ± 0.45
240-250	9 min 30 s	140	63.39 ± 0.46	58.12 ± 0.68	10.78 ± 0.18	0.30 ± 0.1	3.44 ± 0.12

The findings revealed that laminated biscuit baking at 260°C for 5 min and 30 s led to water contents greater than 5% (w.b.). It was observed that by increasing the steam during baking at a constant temperature, the length and width of biscuits increased, and consequently, their thickness decreased. This phenomenon can be explained by the delayed crust formation due to more steam, allowing more time for the biscuits to change shape. Mohd Jusoh et al. (2013) observed that humidified baking significantly reduced bread crust thickness and increased water content, both when the bread was fresh and during storage. They stated that this happened because high humidity in the oven reduces the heat and moisture gradients that normally cause crust formation and moisture loss. It was also observed that increasing the steam causes a rise in surface hairlines (Figure 4.1), which might be attributed to an increase in length and width, putting stress on the biscuits. To avoid hairlines in biscuits, steam injection of 140 mL (140 mL of water in the form of steam) was selected for the baking of laminated biscuits. Although baking at 240°C for 9 min led to the desired density and water content, cracks were observed in biscuits a few days after baking. This occurs mainly due to uneven water distribution in biscuits (D. J. R. Manley, 1998a). Therefore, the baking temperatures of 240°C (on the top part of oven) and 250°C (at the bottom part of oven) were selected to avoid the formation of cracks.

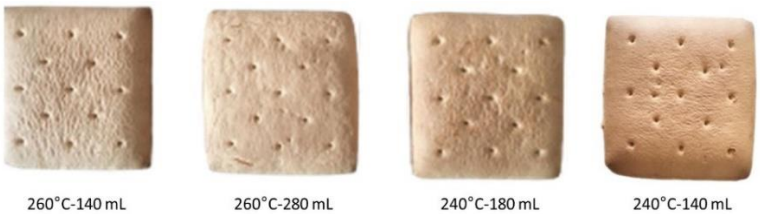

Figure 4.1. Appearance of laminated biscuits (containing LAs) baked under different conditions.

The oven temperature, dew point, and specific humidity registered by HYGROX for baking of laminated biscuits at 240-250°C for 9 min and 30 s is depicted below (Figure 4.2):

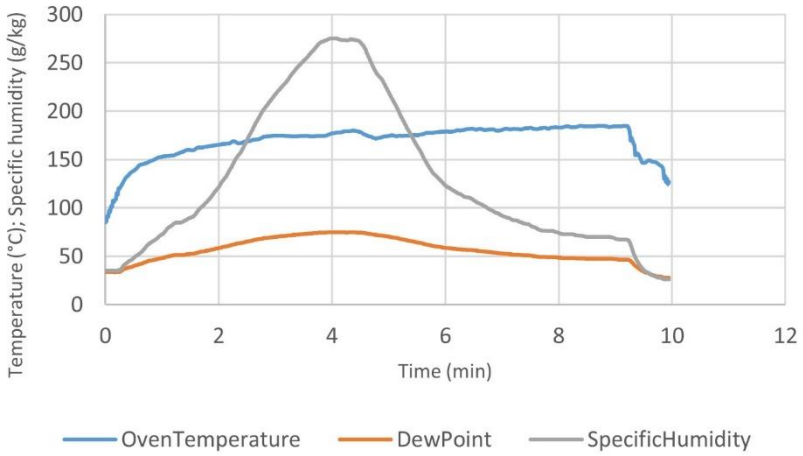

Figure 4.2. Oven temperature, dew point (°C), and specific humidity (g /kg humid air) registered by HYGROX for the baking condition of 240-250°C for 9 min and 30 s for laminated biscuits.

For baking with 140 mL of water in the form of steam, the HYGROX data showed a high specific humidity during the first 4–5 min of the baking cycle, reaching a peak of approximately 250 g /kg. In the early baking stages, when the dough is still expanding and setting, this indicates that the biscuit surface was exposed to moist air. The dew point also rose significantly, reaching around 70°C, indicating that the air was near saturation, thereby slowing down surface dehydration. By delaying the development of a hard crust, the biscuits' surface was able to expand more evenly, reducing surface stress and avoiding the development of hairlines.

Humidity and dew point frequently have a close connection. The dew point is the temperature at which dew or condensation begins to develop, and it is primarily controlled by the moisture in the air. The dew point rises in proportion to the humidity. When the dew point and temperature are the same, the humidity is 100%. If the air temperature is lower than the dew point, condensation will occur, resulting in dew. On the other hand, when the air temperature is above the dew point, the relative humidity falls and the air can store more water vapour. Temperature influences the relation between humidity and dew point. When the temperature lowers, the air can no longer carry as much water vapour, resulting in lower humidity levels. Furthermore, temperature influences the dew point, with greater temperatures resulting in higher dew points. This implies that when the dew point is achieved, the humidity is at its highest level and the air becomes saturated with moisture. As seen in Figure 4.2, the oven temperature is greater than the dew point, causing water vapour to turn into liquid and delaying crust formation, giving the biscuit more time to expand. However, injecting additional steam causes more liquid to form, resulting in explosive growth and biscuit collapse.

4.1.5. Comparison of leavening agents-free (LAs-free) laminated biscuits mixed under CO_2 over-pressure with and without gas flow

LAs-free laminated biscuits properties made under two different mixing conditions are presented below (Table 4.8):

Table 4.8. Properties of LAs-free laminated dough with two CO_2 over-pressure mixing protocols and laminated biscuits.

	length (mm)	Width(mm)	Thickness(mm)	Specific volume of biscuit (cm^3/g)	Dough void fraction after mixing (%)
LAs-free laminated biscuit + CO_2 (protocol A)	62.44 ± 0.77	53.02 ± 0.97	8.03 ± 0.25	2.17 ± 0.47	19.59 ± 0.17
LAs-free laminated biscuit + CO_2 (protocol B)	62.09 ± 0.71	55.72 ± 0.99	7.18 ± 0.26	2.13 ± 0.03	19.82 ± 0.88

A two-sample t-test indicated no significant difference ($p > 0.05$) in the dough void fraction and the specific volume of the baked biscuits between the two studied protocols. To better understand the solubility behavior of gases in laminated biscuit dough, two experimental protocols involving CO_2 injection were compared based on their partial gas pressures in the mixer's headspace.

- Protocol A (no gas flow): CO_2 Injection Without Vacuum

In this protocol, CO_2 was injected into a mixer initially filled with air. This resulted in a partial pressure of CO_2 of 0.5 bar and air at 1 bar. The presence of ambient air meant that the partial pressure of oxygen remained similar to atmospheric levels, thereby supporting gluten network formation.

- Protocol B (gas flow): CO_2 Injection After Vacuum

In contrast to the previous protocol, this protocol involved an initial vacuum step during the first minute of mixing, reducing the pressure in the mixer to 0.1 bar before CO_2 injection. While Protocol B enabled a significantly higher level of CO_2 solubilisation, the initial vacuum step also drastically reduced the O_2 partial pressure:

At 0.1 bar total air pressure:

  - $P\ O_2 \approx 0.21 \times 0.1 = 0.021$ bar
  - $P\ N_2 \approx 0.79 \times 0.1 = 0.079$ bar

Subsequent CO_2 injection increased the total pressure to 1.4 bar, resulting in a partial pressure of CO_2 of 1.4 bar. This represents a +180% increase in CO_2 partial pressure compared to Protocol A:

$$\Delta P_CO2 = ((1.4 - 0.5) / 0.5) \cdot 100 = 180\% \quad \textit{Eq. 4.1}$$

According to Henry's Law, this higher partial pressure directly leads to a greater amount of CO_2 dissolved in the aqueous phase of the dough during mixing.

4.1.6. Freezable water in the laminated biscuit dough

Figure 4.3 shows the fusion energy of the frozen water in the laminated biscuit dough.

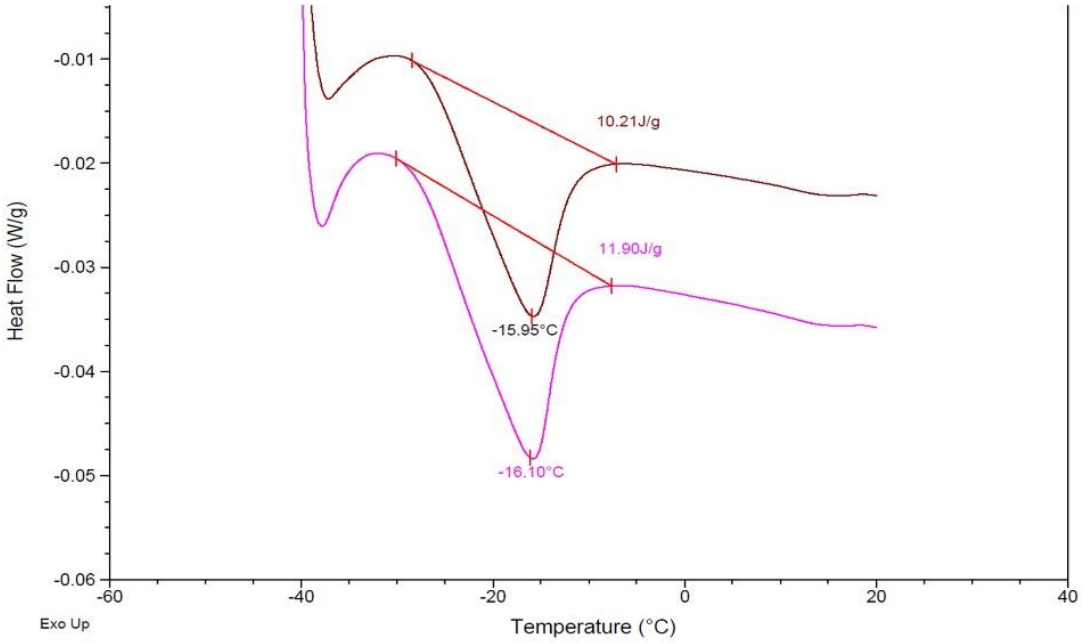

Figure 4.3. Fusion energy of the frozen water in the dough (J/g) determined by DSC; each curve represents one test.

Considering the latent heat of fusion of pure water as 334 J/g, the amount of freezable water in the dough is 0.03 g water/g biscuit dough, indicating that the amount of water available for dissolving CO_2 in the biscuit dough is very small.

4.1.7. Conclusion

Enilia (T55) flour was chosen among the three tested flours because it could make biscuits with a density within the industrial threshold (0–3 g/cm³). The optimal kneading conditions for laminated biscuit dough containing LAs and the LAs-free one under atmospheric pressure and CO_2 over-pressure conditions were 100 rpm for 8 min and 180 rpm for 10 min, respectively. This balanced complete dough development with a high void fraction. Surface cracks were reduced and adequate water content was guaranteed by baking at 240–250°C with 140 mL of water in the form of steam.

The development of gluten may have been hindered by decreased O_2 availability even though Protocol B (vacuum + CO_2 injection) produced higher CO_2 solubility (+180%) as a result of increased partial pressure. There was no visible difference in the specific volume of biscuits or the void fraction of dough between the two CO_2 protocols indicating that dough structure and gas solubility were in equilibrium. This can be attributed to the small amount of freezable water present in the laminated biscuit dough.

4.2. Article 1: Impact of removing leavening agents from laminated biscuits

The results of laminated biscuit dough mixing under CO_2 over-pressure and its impact on biscuit properties are presented in this part. The findings related to this experiment are written in the form of an article and have been submitted to the *Journal of Food Engineering*. In the submitted article, the control biscuit is the conventionally-baked laminated biscuit containing LAs (LB-ABC-CB), NCL refers to the laminated biscuit free from LAs (LF-LB), NCL+CO2 refers to the laminated biscuit free from LAs with CO_2 over-pressure mixing (LF-LB-CO2), and NABC+CO2 refers to the laminated biscuit free from ABC, containing SBC and SAPP, prepared under CO_2 over-pressure mixing (LB-CO2-CB).

Interest in biscuit dough mixing under CO_2 pressure as a strategy to reduce chemical leavening in laminated biscuits

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Abstract

This study examines the potential for reducing the demand for chemical leaveners in laminated biscuits by utilising carbon dioxide (CO_2) in over-pressure mixing. Traditional biscuit formulations mostly use sodium bicarbonate (SBC) and ammonium bicarbonate (ABC) for leavening. SBC needs acid salts like sodium acid pyrophosphate (SAPP) to release full CO_2, while ABC decomposes completely at ~60°C, releasing ammonia that may raise the risk of acrylamide (AA) formation due to pH effects and interactions with asparagine. By replacing ABC and modifying standard formulations, biscuit doughs were mixed under 0.5 bar CO_2 headspace pressure to explore cleaner-label possibilities. When compared to the control, CO_2 mixing significantly increased the dough's void fraction by up to two times and resulted in biscuits that were lighter in colour, which may indicate lower levels of AA. However, these biscuits showed increased hardness and decreased specific volume, especially in formulations entirely free from chemical leaveners. Comparing SBC and over-pressure CO_2 mixing (NABC+CO2) to doughs without chemical leaveners, the former increased porosity and decreased biscuit hardness. Despite the difficulty in achieving the ideal texture, these results show that over-

pressure CO_2 mixing can improve dough aeration and partially offset the use of fewer chemical leaveners. It may be possible to reduce ABC and SBC more successfully while preserving the desired biscuit quality with additional work at higher CO_2 pressures.

Keywords: Laminated biscuits, over-pressure mixing, dough aeration, chemical leaveners, texture

4.2.1. Introduction

Biscuits are mainly comprised of wheat flour, sugar, and fat. They are readily available and the most popular baked foods with a long shelf life (Garcia-Armenta et al., 2017). When stored in moisture-proof containers, they have a lengthy shelf life of at least six months with a typical water content in the range of 4% (w.b.). The attraction among consumers is established by the food's look, sweet taste, ready-to-eat nature, low price, and long shelf life (Manley, 2001, 1998). Controlling the biscuit aeration is a key feature that may influence the texture of the biscuits. Mixing and the use of chemical leaveners are the two major players in this domain.

Mixing conditions, such as mixer type, rotation speed, mixing time, and water content, as well as the ingredients themselves, will affect both the aeration of the dough and its texture. During mixing, the dough undergoes a progressive texturisation accompanied by air entrapment in the form of miniature gas bubbles. The aerated structure of the biscuit is formed by these bubbles, which serve as diffusion nuclei for gases like CO_2 produced during baking by chemical leaveners (Brijwani et al., 2008).

ABC and SBC are the principal chemical leaveners in the biscuit industry. The progressive thermal decomposition of these chemicals during baking produces CO_2 and causes the biscuit dough to expand. ABC, which is a volatile salt, is decomposed during heating from around 60°C; it releases CO_2, ammonia (NH_3), and water, contributing to the expansion of the biscuit's dough. Water and CO_2 are also released when SBC is heated (with decomposition temperature range of above 87-177°C (Wu & Shih, 1993), leaving sodium carbonate as the residual salt. SBC is used to adjust the pH of the dough as well. In biscuits containing SBC, SAPP is a common acid utilised to react with the residual salt and allows full liberation of CO_2 during the baking process. The biscuit's structural integrity may be significantly affected by using too much ABC. As NH_3 has a very strong and disagreeable flavour and odour, it must be completely released during baking. Likewise, an alkaline reaction caused by excessive SBC utilisation produces a yellowish crumb and surface colouration as well as a characteristic known as soda bite (Manley, 2011; Whiteley, 1971).

The increasing focus on clean-label food products is a reflection of consumers' desire to reduce the use of specific chemicals and additives. Research studies indicate that ABC contributes to the enhancement of the AA formation by releasing NH_3, which increases the pH of the dough and makes

an alkaline environment that speeds up the Maillard reaction (Masatcioglu et al., 2014; Palermo et al., 2016). The occurrence of this reaction arises from the heating of reducing sugars and the amino acid asparagine; the presence of NH_3 promotes the formation of AA by increasing the availability of free asparagine (Lo Faro et al., 2022). The benchmark levels for the presence of AA in wheat-based bread and biscuits are 50 µg/kg and 350 µg/kg, respectively (European Commission, 2017).

One strategy to reduce AA, which the International Agency for Research on Cancer has named as a probable carcinogen, is to substitute a non-ammoniagenic chemical leavener for ABC. After intake, AA spreads throughout the brain, liver, breast, heart, and kidneys, where it may disrupt normal cell division, development, metabolism, and reproductive processes. It is changed into glycidamide, which has a high potential to damage DNA, by the body during metabolism. Additionally, long-term low-dose exposure can damage neurotransmission and axonal function (Maan et al., 2022). In a study conducted by Lo Faro et al. (2022), by partially substituting ABC with SBC, reducing the baking temperature, and releasing steam for a specific duration, a 87.2% decrease in AA concentration was achieved compared to the reference biscuits.

Furthermore, concerns about the potential health implications of phosphate-containing compounds like SAPP are raising awareness among consumers and public health advocates. Abd-Elhakim et al. (2018) evaluated the hemato-immunotoxic effects of SAPP and three other food additives. In this investigation, SAPP (12.6 mg/kg) was administered orally to one group of rats for 90 days. A significant leukopenic state was linked to SAPP. The results showed that long-term exposure to high SAPP dosages has immunotoxic and hematotoxic effects and could cause immune suppression.

Mixing and aeration of biscuit dough play a crucial role in the void fraction, which significantly affects the mechanical and fracture properties of the biscuits. The processing, packaging, and structure development of the biscuit depend heavily on these characteristics (Brijwani et al., 2008). Researchers have investigated several methods for dough aeration. Over-pressure mixing is one of the techniques that they have studied. Massey et al. (2001) examined the relationship between the headspace pressure and the gas volume fraction and bubble size in aerated cake batter, finding that these characteristics increase with increasing pressure. Brijwani et al. (2008) observed similar results in biscuit dough. They investigated the effects of various CO_2 and N_2 headspace compositions and pressures on biscuit dough to verify the effects on dough aeration, rheological properties, degassing during sheeting, and the final biscuit texture and physical qualities. According to their findings, higher CO_2 levels and increased pressure significantly boosted the gas content in the dough. As a result, the biscuits baked under these conditions spread more and rose higher, with a softer texture.

Martin et al. (2008) examined the mixing of bread doughs under controlled environments of air and CO_2. They demonstrated how certain aspects of crumb structure could be efficiently controlled by pressure-CO_2 mixing. Unlike Massey et al. (2001), who showed that bubble size increases with pressure, Martin et al. (2008) showed that increased pressure affects the number of bubbles in the crumb. In line with Massey et al. (2008), two processes cause bubble size to increase: the desorption of soluble gases, which occurs when gas moves from the liquid phase to the bubbles, and the increase in bubble size as pressure returns to atmospheric levels. This results in an additional volume increase as the gas desolubilises when pressure is reduced. Sadot et al. (2017) examined the effect of pressure variation on bread dough aeration using a spiral dough mixer that had temperature and overhead pressure control between -960 mbar and +500 mbar. According to their findings, dough aeration and the number of spiral tool rotations were directly related. A larger pressure drop resulted in a longer time to reach equilibrium, and the distrainment rate was slower with the highest pressure drop.

Previous studies suggest that over-pressure mixing offers a promising alternative for dough aeration, potentially replacing traditional methods that rely on chemical leaveners. When considering over-pressure mixing, the choice of gas used plays an important role in the dough's properties and the final product. The main constituent of air, nitrogen (N_2), is a poorly soluble gas that does not chemically interact with the ingredients in the dough. In contrast, CO_2 is highly soluble in both water and fat, with almost similar levels of solubility. As the temperature increases, the solubility of CO_2 in water decreases, while its solubility in fat increases (Jakobsen et al., 2009). For instance, the solubility of CO_2 in water decreases from 0.3346 g/100 mL H_2O at 0°C to 0.0576 at 60°C at 101.3 kPa (partial pressure) (Lange, 2013). Furthermore, the incorporation of CO_2 in the mixing process can influence the texture and structure of biscuits. Brijwani et al. (2008) observed that biscuits mixed with CO_2 or a CO_2-N_2 mixture showed increased thickness compared to those mixed with N_2. Notably, biscuits mixed with CO_2 were softer, while those mixed with the CO_2-N_2 mixture were stiffer, and biscuits mixed with N_2 produced the hardest textures. These findings suggest that CO_2 influences the dough's void fraction and structure in ways that may be beneficial for biscuit production.

Based on these findings, the current study aims to evaluate the potential of using 100% CO_2 at a pressure of 0.5 bar in the headspace of a prototype mixer as a means of replacing chemical leavener ABC in biscuit dough. It is hypothesised that over-pressure mixing with CO_2 may enhance the dough's void fraction, increase biscuit volume, and improve the biscuit's final texture. The proposed hypothesis was tested on: a control biscuit dough, containing both ABC, SBC, and SAPP; two biscuit doughs free of chemical leaveners, prepared both with and without CO_2 mixing; and a biscuit dough free of ABC but containing SBC, mixed under CO_2 pressure. This was done to evaluate the impact of CO_2 over-pressure mixing on the properties of laminated biscuit dough.

The introduction of over-pressure mixing using CO_2 is expected to increase the void space in the dough, as gas bubbles serve as nucleation sites for structure development during baking. Additionally, mixing in an environment high in CO_2 may promote biscuit expansion resulting from dissolved CO_2 released during baking, facilitating the rising process in the oven.

4.2.2. Materials and methods

4.2.2.1. Materials

Four types of biscuit dough were prepared using wheat flour type T55 (14.27% water content, 9.60% protein, 14.3 UCD damaged starch, and 0.58% ash w.b.) (Enilia, France), butter with 82% fat matter (Président, France), icing sugar (Béghin Say, France), salt, water, glucose syrup (Vahiné, France), ABC (Brenntag, Germany), SBC (Brenntag, Germany), and SAPP10 (Budenheim, Germany). The SAPP10 has been selected as a slow-acting agent, releasing just 10% of the CO_2 from SBC during dough mixing. The formulation of laminated biscuit dough is presented in Table 4.9. It must be noted that all doughs had water content of the same order of magnitude (22%, w.b.).

4.2.2.2. Mixing and dough preparation

Before mixing, glucose syrup was diluted in a small volume of water. ABC was also dissolved in a portion of water. The remaining water was combined with sugar and salt and thoroughly mixed to prevent agglomeration. All ingredients were then simultaneously introduced into the mixer with a single spiral blade.

A prototype batch mixer (Figure 4.4) with a bowl volume of 11 L and a specified capacity of up to 5 kg of dough (VMI, St Hilaire de Loulay, France) was used for mixing. A water jacket that surrounds the mixing bowl regulates dough temperature. The mixer is moving by rotating both the tool and the satellites, while the bowl stays static. The mixer rotation speed was determined experimentally by selecting the speed that resulted in the highest void fraction in the dough. During the mixing process, an infrared temperature sensor was used to measure dough temperature. The temperature in the case of CO_2 mixing was set at 25°C to enhance the solubility of this gas in water, given that the dough contains more water than fat. For each test, batches of 3 kg of dough were prepared. As part of a washout procedure, air was pumped into the mixer headspace for a minute before the pressure was applied. In order to give the dough sufficient oxygen renewal, as it consumes oxygen through solubilisation, a moderate airflow was maintained through the mixer's headspace when mixing under atmospheric conditions. In the absence of ABC, CO_2 was added at a gauge pressure of 0.5 bar (above atmospheric pressure) to improve the dough aeration. The biscuit dough mixing protocol is shown in Table 4.10.

Table 4.9. Laminated biscuit dough recipes; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing)

	Mass of ingredients (g) per 100 g of flour (w.b)			
Ingredients	Control	NCL	NCL + CO2	NABC + CO2
Flour	100	100	100	100
Butter	5.53	5.53	5.53	5.53
Icing sugar	31.85	31.85	31.85	31.85
Water	20.77	20.77	20.77	20.77
ABC	1	-	-	-
SBC	0.64	-	-	0.64
SAPP 10	0.40	-	-	0.40
Salt	0.94	0.94	0.94	0.94
Glucose syrup	2.23	2.23	2.23	2.23
Total	**163.36**	**161.32**	**161.32**	**162.36**



Figure 4.4. Prototype batch mixer VMI.

4.2.2.3. Biscuit dough sheeting and shaping

The biscuit dough was laminated using a RONDO dough sheeter (RONDO, Germany) after a 30-minute rest at 27°C. Lamination was performed in five steps, with nips between the rolls of 10, 8, 6, 4, and 2 mm. The laminated dough was then shaped manually using a 60 × 60 mm rectangular cutter equipped with needles. Shaping occurred 1 minute after lamination to reduce shrinkage. The biscuits were placed on an oven rack for baking. A total of 28 biscuits were baked at a time.

Table 4.10. Biscuit dough mixing protocol.

Control	NCL	NCL+CO2	NABC+CO2
Solutions: Icing sugar + Salt + Water ABC + Water Glucose syrup + Water	Solutions: Icing sugar + Salt + Water Glucose syrup + Water	Solutions: Icing sugar + Salt + Water Glucose syrup + Water	Solutions: Icing sugar + Salt + Water Glucose syrup + Water
For each type of dough, the prepared solutions are combined to form a single solution, referred to as "Solution A"			
Flour + SBC + SAPP10 + Butter + Solution A (all in the mixer)	Flour + Butter + Solution A (all in the mixer)	Flour + Butter + Solution A (all in the mixer)	Flour + SBC + SAPP10 + Butter + Solution A (all in the mixer)
1 min air flushing (Tool: 50 rpm; satellite: 5 rpm) 1 min air injection (Tool: 100 rpm; satellite: 5 rpm) 8 min air injection (Tool: 100 rpm; satellite: 10 rpm) Temperature: 30°C		1 min CO_2 flushing (Tool: 50 rpm; satellite: 5 rpm) 2 min CO_2 injection (Tool: 100 rpm; satellite: 5 rpm) 10 min CO_2 injection (Tool: 180 rpm; satellite: 10 rpm) Temperature: 25°C	
30 min dough rest at 27°C			

(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO2 mixing; NABC+CO2: no ammonium bicarbonate +CO2 mixing).

4.2.2.4. Baking

The biscuits were baked in a deck oven (MIWE, Germany) at 240°C (top) and 250°C (bottom) for 9 min 30 s. In the case of NCL dough, baking was performed for 10 min in order to prevent the formation of cracks on biscuits, which form due to nonhomogeneous water distribution in dough pieces during baking. For each batch, eight biscuits were selected and marked for further analysis. Experiments were conducted in triplicate. To mimic industrial baking conditions (based on recording done in industry), 140 mL of water was injected into the oven as saturated steam during the first 2 min of baking. This steam delayed the formation of the crust, allowing the biscuits sufficient time for water evaporation and resulting in a better oven rise. After baking, the biscuits were left to cool at the ambient temperature for 30 minutes. After cooling, they were placed in a sealed plastic bag for further analysis.

4.2.2.5. Analysis of dough and biscuits

4.2.2.5.1. Dough pH

The pH of biscuit dough was measured at 25°C with a solid electrode (WTW 3110, Xylem Analytics, Germany).

4.2.2.5.2. Void fraction of gas in dough

Aeration of biscuit dough was assessed through void fraction measurements by using apparent density (ρ_{app}) and gas-free density (ρ_{gf}) of dough. The procedure used by Sadot et al. (2017), with some modifications, was applied to measure the apparent density, also called bulk density; this procedure is based on Archimedes' principle. In this method, dough is weighed in air, then immersed in a liquid of known density and weighed again; from the difference in the weights, and knowing the density of the liquid, the dough density can be calculated. Canola oil was used as the immersion liquid. The void fraction of gas content (∅) of the dough was calculated according to Equation (*Eq. 4.2*).

$$\emptyset = 1 - \frac{\rho_{app}}{\rho_{gf}} \qquad \textit{Eq. 4.2}$$

The gas-free dough density was calculated by using the volume fraction (X_i^v) and the density (ρ_i) of all ingredients according to Equation (*Eq. 4.3*) (Sahin & Şumnu, 2006).

$$\rho_{gf} = \sum \rho_i \cdot X_i^v \qquad \textit{Eq. 4.3}$$

4.2.2.5.3. Morphology and apparent density of biscuits

The thickness, length, and width of eight baked biscuits were measured with a digital calliper (Mitutoyo, Japan) the day after baking. Thickness was defined as the average of the thickness at three points. The specific volume of biscuit (SV_{bis}) was calculated based on Equation (*Eq. 4.4*) as follows:

$$SV_{bis} = \frac{length \cdot width \cdot thickness}{W_{baked}} \qquad \textit{Eq. 4.4}$$

where W_{baked} is the weight of a baked biscuit.

4.2.2.5.4. Biscuit porosity

Biscuit porosity (P) was calculated according to Equation (*Eq. 4.5*).

$$P = 1 - \frac{\rho_{app,bis}}{\rho_{solid}} \qquad \textit{Eq. 4.5}$$

where $\rho_{app,bis}$ is the apparent density (bulk density) of biscuit (pores included), which is defined as the mass/volume ratio, and it is the inverse of SV_{bis}. ρ_{solid} is the solid density of the pure solid without pores measured using a helium pycnometer, 10 times for each biscuit (Accupyc 1330, Micromeritics, USA). The porosity value is expressed as a percentage.

4.2.2.5.5. Water content of biscuits

The water content of the biscuits was determined following the AACC Method 44-15.02. A sample of 3 g (w.b.) powdered biscuit was placed in an aluminium cup and dried in a drying cabinet at 105°C for 24 h. After drying, the sample was placed in a desiccator for 10 min before being weighed. Measurements were performed in triplicate.

4.2.2.5.6. Colour of biscuits

The colour of five biscuits was assessed 24 h after baking with a colorimeter (Konica Minolta, France), targeting the centres and edges of both the top and bottom surfaces. The colour measurements were based on the CIE system parameters: L^* (lightness), a^* (redness), and b^* (yellowness). The difference in colour (ΔE) between the control (with notation 1) and reformulated biscuits (with notation 2) was determined using Equation (*Eq. 4.6*).

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad \textit{Eq. 4.6}$$

4.2.2.5.7. Texture of biscuits

Hardness analysis of biscuits was conducted using a texture analyser (AMETEK-TA, USA) coupled with a load of 1 kN. The three-point bending test was applied to quantify the hardness. The measurement pre-test speed of 1 mm/s, test speed of 5 mm/s, and a distance of 10 mm were applied. The span between the supports was 35 mm. The force-displacement plots were analysed for peak breaking force (N). Textural attributes were measured in five samples. It must be noticed that this test was performed 7 days after baking biscuits to ensure there were no cracks on their surfaces.

4.2.2.5.8. Statistical analysis

Data validation was performed using one-way analysis of variance (ANOVA) in STATGRAPHICS 19 software. Significant difference analysis was carried out at the 95% confidence interval. ANOVA was followed by Fisher's Least Significant Difference (LSD) post hoc test.

4.2.3. Results and discussion

4.2.3.1. Dough properties

4.2.3.1.1. Dough pH

Variations in pH of biscuit doughs were observed depending on the formulation (Figure 4.5). The control dough pH (7.23 ± 0.01) was noticeably higher than that of the other doughs. Nevertheless, there was no significant difference between NCL and NCL+CO2, but NABC+CO2 displayed a significant difference from NCL and NCL+CO2. Although CO_2 was dissolved in the biscuit dough through pressure mixing, which produced acidification, it was observed that this decrease in pH is mainly due to the removal of ABC, as there was not a significant difference between the pH of NCL and NCL+CO2. As the pH level significantly affects the mechanisms underlying the Maillard reaction, there might be less AA in biscuits without ABC (NCL, NCL+CO2, and NABC+CO2) than in those with ABC (control), as the formation of AA in food decreases at lower pH values (Govindaraju et al., 2024; Khorshidian et al., 2020).

4.2.3.1.2. Void fraction of gas in dough

The biscuit dough's void fraction at the post-mixing and post-sheeting processing stages was measured (Figure 4.6). All dough formulations showed statistically significant variations in the void fraction after the mixing stage according to ANOVA analysis. NABC+CO2 and NCL+CO2 doughs had void fractions of 21.06 ± 0.74% and 19.59 ± 0.17%, respectively. This is 2.2 and 2.0 times higher than that of the NCL dough (9.62 ± 0.31%). The void fraction of 14.47 ± 0.86%, which was roughly 1.5 times higher than NCL, was observed in the control dough that contained ABC and SBC. The void fraction of the control and NABC+CO2 doughs differed significantly at the sheeting stage. As SBC was included in the NABC+CO2 formulation, there was also a significant difference in the void fractions of the NABC+CO2 and NCL+CO2 doughs. According to these results, adding CO_2 improves gas entrapment during mixing, which is in line with Brijwani et al., (2008) earlier findings about CO_2's high solubility in dough. Following sheeting, a significant decrease in the void fraction was observed in all samples, suggesting that the dough is compressed during the sheeting process, leading to degassing and a reduction in gas volume. Even though this decrease might result in a denser dough, the addition of chemical leaveners, like ABC and SBC, during baking makes up for gas loss by producing more gas, which lowers the density of the finished product.

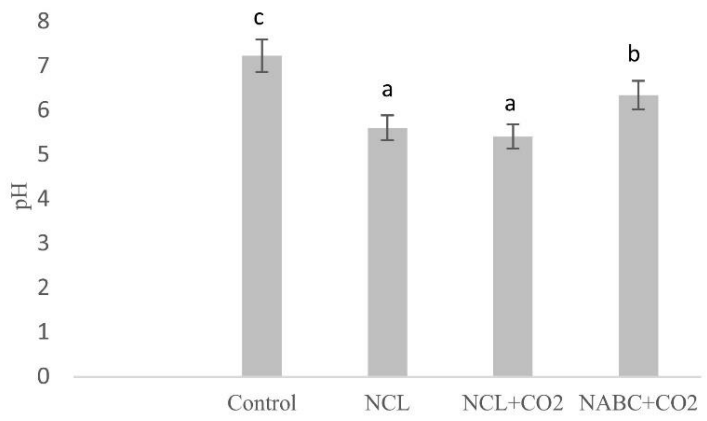

Figure 4.5. pH of biscuit doughs at 25°C;(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval).

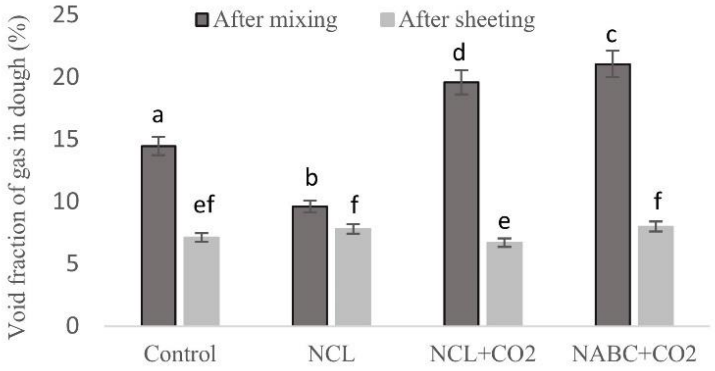

Figure 4.6. Void fraction of gas of the biscuit doughs; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval).

4.2.3.1.3. Morphology, apparent density, and porosity of biscuits

The biscuits were formed by shaping the dough with a 60×60 mm cutter after it had been laminated. The biscuits' post-baking measurements, specific volume, and porosity were evaluated (Table 4.11). In light of these findings, biscuits shrank after baking. Biscuits shrink when they are laminated and baked. Shrinkage may also be connected to the gluten network development and mixing stage. Avoiding this phenomenon entirely is not possible. The dough's characteristics and its behaviour during baking cause it to shrink significantly in length. A moisture gradient develops during the drying process of the dough, which causes the protein and starch structures to shrink. The dough's length and, to a lesser degree, its width are both impacted by this shrinkage. The quality of the flour and the characteristics of the dough have the biggest effects on size and shape changes (D. Manley, 2011; D. J. R. Manley, 1998c; Singhal et al., 1997; Whiteley, 1971).

After baking, biscuit dough pieces thicken. The primary cause of these modifications is temperature, which affects several aspects of the recipe, as well as the dough itself. The dough's density drastically drops as CO_2 gas is released from chemical leaveners or trapped CO_2 in the process of mixing under gas pressure, and water vapour bubbles form and expand. This gives the biscuit its delightful texture by forming a light, airy structure.

Except for biscuits made from NCL and NCL+CO2 doughs, which showed no significant difference in length, all biscuit types showed statistically significant variations in length according to the results. All biscuit varieties showed notable variations in width. Additionally, there were notable differences in thickness among all samples. The combined presence of both ABC and SBC is probably why the control biscuits performed better than any other type. The fact that biscuits made with NABC+CO2 dough were thicker than those made with NCL+CO2 suggests that SBC helps to increase thickness. The NCL+CO2 biscuits were the thinnest, indicating that CO_2 by itself has little effect on improving biscuit thickness and texture when used as the only leavener.

All biscuit types had significantly different specific volumes. The NABC+CO2 biscuits came in second, with the control biscuits showing the highest specific volume. Even though the specific volume of the NABC+CO2 biscuits was much higher than that of the NCL+CO2 biscuits, the difference was only 1.27 times. Every comparison revealed statistically significant variations in porosity, which is directly related to specific volume. These results indicate that although dough mixed under CO_2 pressure has a higher initial void fraction, the control formulation, which contains ABC, SBC, and SAPP, allows for a longer-lasting gas release during baking, which leads to more expansion and a thicker finished product. Under high pressure, the high solubility of CO_2 is further enhanced. When dough is mixed under high pressure, a significant amount of CO_2 can dissolve in the dough. The gas is released, and bubbles form when the pressure is subsequently lowered to atmospheric levels because CO_2 becomes less soluble (Brijwani et al., 2008).

4.2.3.1.4. Water content of biscuits

Figure 4.7 illustrates the control and reformulated biscuits' water contents. Even though the water content of all doughs had a similar magnitude (22% w.b.), nearly all pairs showed statistically significant differences, except the control and NCL+CO2 biscuits. Notably, the NCL+CO2 biscuits started to develop surface cracks a few days after baking, most likely because of an uneven water distribution. They have fewer voids for moisture to escape during baking because of their lower specific volume and porosity, which causes water to be retained and dispersed unevenly. The longer baking time of the NCL+CO2 biscuits may have contributed to their lower water retention compared to the control biscuits. The NABC+CO2 biscuits, on the other hand, showed less water content than the NCL+CO2

biscuits. This is probably because of their higher porosity, which facilitates more effective water evaporation during baking.

Table 4.11. Dough pieces and biscuit attributes.

	Thickness,BB (mm)	Length, AB (mm)	Width, AB (mm)	Thickness, AB (mm)	Specific volume (cm^3/g)	Real density (g/cm^3)	Porosity
Control	2.68 ± 0.21^a	66.10 ± 0.51^a	61.43 ± 0.93^a	10.18 ± 0.16^a	3.43 ± 0.07^a	1.60 ± 0.00^a	0.82 ± 0.01^a
NCL	2.53 ± 0.18^a	61.18 ± 1.16^b	45.62 ± 3.66^b	8.46 ± 0.43^b	1.61 ± 0.04^b	1.59 ± 0.00^a	0.61 ± 0.01^b
NCL+CO2	2.61 ± 0.11^a	62.44 ± 0.77^c	53.02 ± 0.97^d	8.03 ± 0.25^d	2.17 ± 0.47^d	1.59 ± 0.00^a	0.72 ± 0.01^d
NABC+CO2	2.79 ± 0.15^a	64.06 ± 0.59^c	58.93 ± 0.93^c	8.82 ± 0.39^c	2.76 ± 0.07^c	1.59 ± 0.00^a	0.77 ± 0.01^c

(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing; BB: before baking; AB: after baking); different letters in the same column indicate significant differences (95% confidence interval).

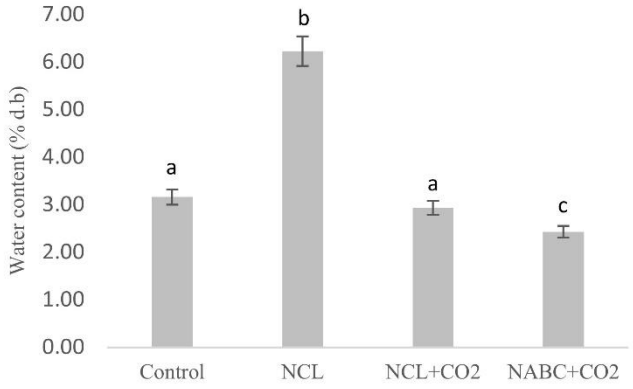

Figure 4.7. Water content of biscuits; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing); bars with the same letter are not significantly different (95% confidence interval).

4.2.3.1.5. Colour of biscuits

To account for possible differences between the upper and lower surfaces, as well as between the central and peripheral regions, the colour of the biscuit crust was evaluated at five different points. The L*, a*, and b* values, as well as the colorimetric distances for the control and modified dough biscuits, were measured (Table 4.12). The NCL biscuits were lighter in colour than the other samples, most likely because ABC, a substance known to aid in biscuit browning through the Maillard reaction, was absent. The biscuits from the NCL+CO2 and NABC+CO2 doughs, respectively, displayed the second and third lightest colour profiles. Differences in biscuit thickness and uneven moisture evaporation during baking could be the cause of variations in colour measurements at various points.

In addition, according to predetermined thresholds, the ΔE value between sample pairs is used to determine whether the human eye can detect a colour difference. In particular, the colour difference is imperceptible if the ΔE < 0.2. While 0.2 < ΔE < 0.5 indicates a very slight difference, 0.5 < ΔE < 1.5 indicates a slight but noticeable variation, 2 < ΔE < 3 indicates a barely noticeable difference, while 3 < ΔE < 6 indicates a distinctly noticeable colour change. Variations between 6 and 12 are regarded as significant (Lo Faro et al., 2022). A noticeable colour difference was observed at all the points. Biscuits made from different doughs are depicted in Figure 4.8.

Gökmen et al. (2008) used computer-based visual image analysis algorithms to create a predictive model for AA in traditional baked cookies. With biscuit browning measured by L*, a*, and b* values, they found a strong positive correlation (r = 0.946, p < 0.01) between AA formation and biscuit browning intensity. Given that the reformulated biscuits exhibit a lighter colouration than the control, there might be less AA concentration in the reformulated biscuits.

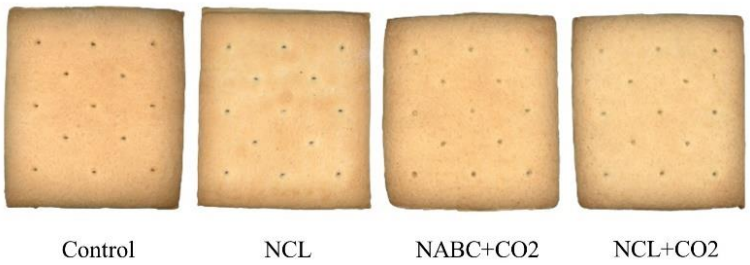

Figure 4.8. Biscuits made from the control and reformulated doughs; (NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO_2 mixing; NABC+CO2: no ammonium bicarbonate +CO_2 mixing).

Table 4.12. Biscuits' colour values and colorimetric distances between control and reformulated biscuits.

Spot/Type of dough	L*	a*	b*	ΔE
Upper left				
Control	62.83 ± 1.37^{a}	10.20 ± 0.17^{a}	29.04 ± 0.61^{a}	0
NCL	70.10 ± 1.04^{b}	7.90 ± 0.49^{b}	32.05 ± 0.67^{b}	1.42
NCL+CO2	66.13 ± 1.70^{ab}	10.54 ± 0.66^{a}	31.82 ± 0.40^{b}	3.77
NABC+CO2	61.71 ± 1.86^{a}	10.12 ± 0.79^{a}	30.03 ± 0.76^{c}	1.49
Upperright				
Control	62.91 ± 0.69^{a}	10.18 ± 0.22^{a}	28.80 ± 0.38^{a}	0
NCL	69.74 ± 1.63^{b}	8.01 ± 0.90^{b}	31.86 ± 0.73^{b}	2.06
NCL+CO2	67.16 ±1.91^{ab}	10.29 ±0.46^{a}	31.98 ±0.52^{b}	5.31
NABC+CO2	63.17 ± 1.07^{a}	10.04 ± 0.60^{a}	30.69 ± 0.73^{b}	1.91
Center				
Control	61.92 ±0.19^{a}	10.47 ± 0.25^{a}	28.82 ± 0.31^{a}	0
NCL	70.60 ± 0.132^{b}	7.42 ± 0.27^{b}	31.98 ± 0.39^{b}	2.95
NCL+CO2	68.09 ±1.79^{ab}	10.33 ± 0.64^{a}	32.12 ± 0.41^{b}	7
NABC+CO2	63.03 ±1.65^{a}	10.12 ± 0.88^{ab}	30.61 ± 0.76^{c}	2.14

Spot/Type of dough	L*	a*	b*	ΔE
Bottom left				
Control	62.23 ± 0.69^{a}	10.26 ± 0.18^{a}	28.90 ± 0.24^{a}	0
NCL	70.91 ± 1.32^{b}	7.25 ± 0.48^{b}	31.46 ± 0.78^{b}	3.03
NCL+CO2	65.90 ± 1.65^{b}	10.72 ± 0.43^{a}	31.70 ± 0.42^{b}	4.64
NABC+CO2	62.85 ± 1.71^{a}	9.82 ± 0.74^{a}	30.11 ± 0.71^{b}	1.43
Bottom right				
Control	61.13 ± 2.24^{a}	9.99 ± 0.32^{a}	28.37 ± 0.88^{a}	0
NCL	71.21 ± 0.92^{b}	7.24 ± 0.43^{b}	31.77 ± 0.50^{b}	4.18
NCL+CO2	67.17 ± 1.38^{c}	10.33 ± 0.43^{a}	31.80 ± 0.16^{b}	6.95
NABC+CO2	63.50 ± 1.81^{a}	9.88 ± 0.65^{a}	30.47 ± 0.23^{c}	3.17

(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO2 mixing; NABC+CO2: no ammonium bicarbonate +CO2 mixing); different letters in the same column at each spot indicate significant differences (95% confidence interval).

4.2.3.1.6. Texture of biscuits

Five samples of biscuits were tested for hardness using the three-point bending test. The findings (Table 4.13) show that the control biscuits had lower hardness than the other formulations.

This is probably due to the presence of ABC and SBC in the dough, which enhanced the texture. In terms of hardness, NCL+CO2 biscuits came in second, while the NCL biscuits were noticeably harder than all other formulations. NCL biscuits were 3 times harder than control biscuits, while NCL+CO2 and NABC+CO2 biscuits were 2.7 and 1.4 times harder than control biscuits, respectively. The correlation found between specific volume (Table 4.11) and biscuit hardness values is consistent with previous studies, such as those conducted by Mouneim et al. (2012) and Besbes et al. (2013) for bread and Palier et al.(2022) for cake.

Table 4.13. Hardness of biscuits.

Dough formulation	Hardness (N)
Control	27.56 ± 3.57^{a}
NCL	87.20 ± 3.33^{b}
NCL+CO2	75.73 ± 5.72^{d}
NABC+CO2	38.35 ± 1.62^{c}

(NCL: no chemical leavener; NCL+CO2: no chemical leavener +CO2 mixing; NABC+CO2: no ammonium bicarbonate +CO2 mixing); different letters in the same column indicate significant differences (95% confidence interval).

4.2.4. Conclusion

The findings showed that biscuits made without chemical leaveners and CO_2 were denser and firmer, whereas incorporating CO_2 through over-pressure mixing improved expansion and texture. The NABC+CO2 biscuits, which combined CO_2 over-pressure mixing with SBC and SAPP10, showed reduced

hardness and improved structure, highlighting the benefits of both physical and chemical leavening. However, the results also indicated that CO_2 over-pressure mixing could not match the performance of chemical leaveners, as the resulting biscuits were still harder than the control. Future work should explore alternatives or optimisations to CO_2 over-pressure mixing—such as modifying the mixing equipment—and include AA quantification and sensory analysis to assess consumer acceptance of the reformulated products.

4.2.5. Author contributions

Conceptualisation, Alain Le-Bail; Methodology, Maryam Valizadeh, Alain Le-Bail; Formal analysis, Maryam Valizadeh; Data curation, Maryam Valizadeh, Alain Le-Bail, Sébastien Curet, Cécile Rannou; writing—original draft preparation, Maryam Valizadeh; writing—review and editing, Maryam Valizadeh, Alain Le-Bail, Cécile Rannou, Sébastien Curet; Supervision, Alain le-Bail, Sébastien Curet, Cécile Rannou; Project administration and funding acquisition, Alain Le-Bail; validation, Alain Le-Bail, Sébastien Curet, Cécile Rannou, Marie-Luce Lebriquer, Gilles Bertheau.

4.2.6. Acknowledgment

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4.2.7. References

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4.3. Microwave baking of biscuits as an alternative to conventional baking

This part of the results discusses the microwave baking of laminated and rotary biscuits containing LAs (LB-ABC and RB-ABC), including the use of combined microwave-infrared baking to improve final product colour. Additionally, the energy consumption for microwave baking of biscuits is discussed, with particular attention to the microwave oven efficiency.

4.3.1. Dielectric properties of the dough

The dielectric properties of materials are important for assessing their interactions with electromagnetic energy in microwave baking. The dielectric properties of laminated and rotary biscuit dough at 23°C are presented below (Table 4.14):

Table 4.14. Dielectric properties of biscuit doughs at 2.45 GHz and 23°C.

Type of Biscuit	Applied force (N)	f (MHz)	ε'	ε"	tan(δ)
LB-ABC	30	2450	11.15 ± 0.3	5.11 ± 0.22	0.46 ± 0.01
RB-ABC	30	2450	7.47 ± 0.24	2.5 ± 0.14	0.34 ± 0.01

(LB-ABC: laminated biscuit dough containing ABC, SBC, and SAPP 10; RB-ABC: rotary biscuit dough containing ABC, SBC, and SAPP 10).

It was observed that both $\varepsilon'$and ε'' were smaller in the rotary biscuit dough, which had a lower dough hydration compared to the laminated biscuit (~ 16% w.b. vs. ~ 22% w.b.). Since these two values show how well biscuit dough stores electromagnetic energy and how well it converts electromagnetic energy into heat, it could be concluded that laminated biscuit dough, which had a higher dough hydration, ~ 22% (w.b.), showed higher dielectric properties. The dissipation factor, $\tan\delta$, was also higher in laminated biscuit dough compared to rotary biscuit dough, indicating that more microwave energy could be converted to heat. This finding is in accordance with the findings of Zhang et al. (2019).

The improved dielectric properties of the laminated biscuit dough can be explained by its higher water content compared to the rotary biscuit dough. The main component of most food products is water. Due to predominance of dipole rotation phenomena at 2450MHz—which results from the presence of positively charged hydrogen atoms and a negatively charged oxygen molecule—water is the primary component that interacts with microwaves (Bou-Orm et al., 2021). Similarly, another study on chickpea

cake batter confirmed the high dielectric properties of water compared to other ingredients in powder form (Alifakı & Şakıyan, 2017). The dielectric properties of the cake batter ingredients at 2450 MHz studied in this research are presented below (Table 4.15):

Table 4.15. Dielectric properties of a cake batter at 2450 MHz and 25°C; adapted from (Alifakı & Şakıyan, 2017).

Ingredient	Dielectric constant	Dielectric loss factor
Baking powder	1.7144 ± 0.3070	0.0355 ± 0.0100
Sugar	1.5942 ± 0.1200	0.0024 ± 0.0007
Water	70.4842 ± 2.2400	9.2665 ± 0.1800
Milk powder	1.6816 ± 0.2100	0.0365 ± 0.0120
Salt	1.9540 ± 0.0040	0.0410 ± 0.0030
Wheat flour	2.0722 ± 0.1900	0.1396 ± 0.0320
Egg white powder	1.6656 ± 0.2200	0.0257 ± 0.0045
Shortening	4.4570 ± 0.0290	0.6330 ± 0.0055

4.3.2. Optimal sample position for microwave baking of the laminated and rotary biscuits

The microwave reflection coefficient, S11, was measured using a vector network analyser connected to the microwave cavity as already described in the Materials and Methods. Different positions for the biscuit dough on top of the PTFE support plate were investigated to find the lowest microwave reflected power. For multimode microwave cavities, such as household microwave ovens, boundary reflections of incident waves cause standing wave patterns to form inside the cavity. As a result, there are spatially different electromagnetic field intensities with nodes and anti-nodes of the electric field. High electric field intensity at the optimum location of the sample potentially improves the microwave power absorption, in turn, improving heating efficiency.

Low values of S11 (in dB) indicate low microwave reflected power. Different dough positions within the microwave cavity were examined, and it was observed that S11 at 2.45 GHz varied greatly with position — ranging from –7.81 dB to –9.74 dB for laminated biscuits, and from –4.44 dB to –4.81 dB for rotary biscuits — at different tested positions, when the tests were carried out without a resonator. The role of a dielectric resonator is explained in the Materials and Methods Section 3.1.2. Similar observations on the impact of sample position in the microwave oven on the energy absorption by the sample in the cavity were reported in the literature.

A similar test was applied to the biscuit dough pieces with a ceramic resonator placed next to the dough sample. It was observed that S11 varied from –16.62 dB to –21.87 dB for laminated biscuits, and from –15.98 dB to –25.95 dB for rotary biscuits, depending on the tested position. These results indicate that the presence of a ceramic resonator can highly improve energy absorption by the biscuit dough pieces. This aligns with the findings of Basak and Rao (2010). Pork Luncheon rolls and white pudding were two pork products used in the study to model how heat is transfered through the meat when it is placed on ceramic plates (alumina and silicon carbide). The findings demonstrated the significant influence of ceramic plates on the formation of hot spots, which can occur on the meats surface in the middle or on the side that is exposed to the plate (Basak & Rao, 2010).

In another study, Rattanadecho and Klinbun (2010) investigated the microwave heating process of dielectric materials placed in a rectangular waveguide with a resonator. Based on their results, the inserted resonator had a strong effect on the uniformity of temperature distribution in a saturated porous packed bed. Greater power absorption occurred within the saturated medium due to standing waves formed by reflections from the resonator inside the processed material.

In a complementary study applied to non-food samples, resin pellets and ethylene glycol**,** researchers demonstrated the development of a microwave reactor based on a cylindrical resonator that has real-time dielectric property monitoring. The reactor was able to monitor variations in resonance frequency and transmission loss as markers of moisture content and physical state while drying resin pellets and heating ethylene glycol by using a variable-frequency solid-state microwave source. By improving material characterisation and heating control, this method showed how resonator-based cavities can facilitate more focused and effective microwave processing (Miyakawa et al., 2021).

Therefore, to ensure more efficient and consistent heating through improved energy coupling, the sample position with the lowest S11 value (the most negative dB) at 2.45 GHz was chosen as the optimal baking position (Figure 4.9 and Figure 4.10).

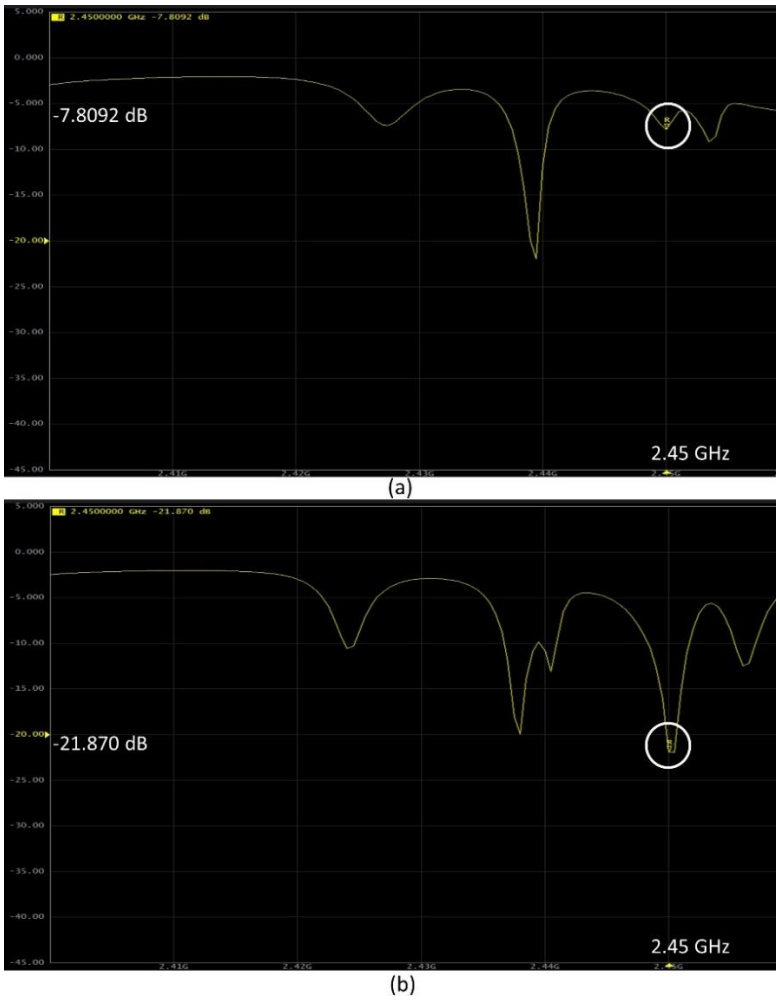

Figure 4.9. S11 value in dB (a) without and (b) with a ceramic resonator at the optimal position for a laminated biscuit; the X-axis represents the microwave frequency in GHz, and the Y-axis represents S11 in dB.

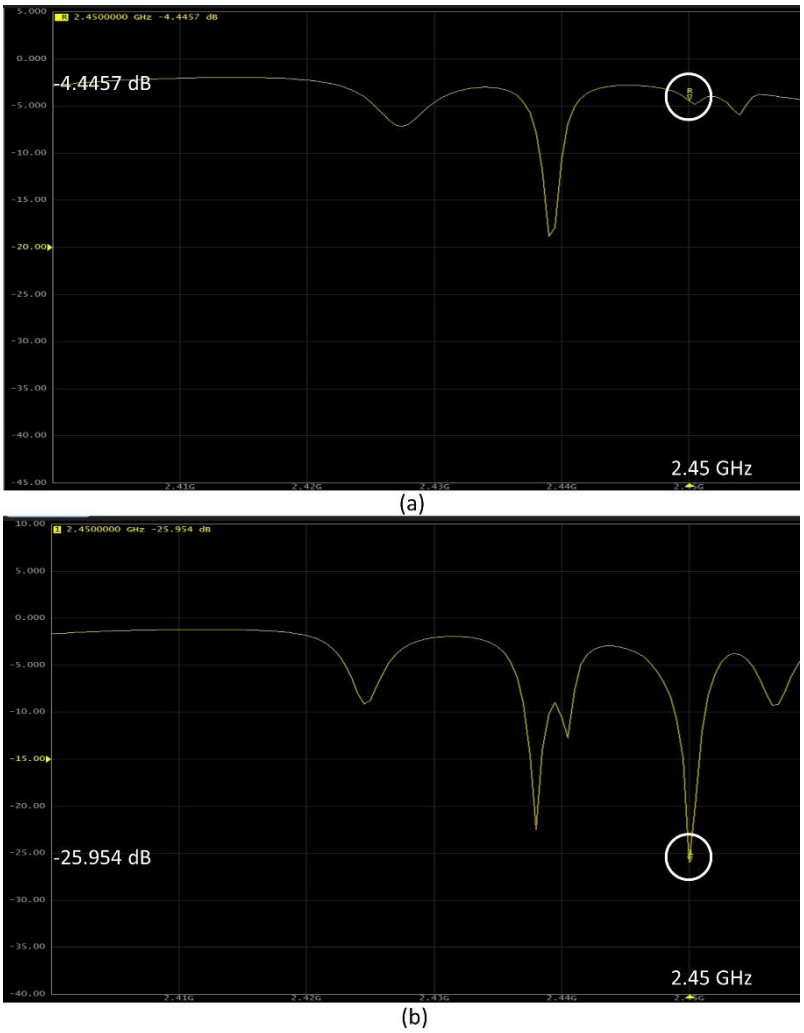

Figure 4.10. S11 value in dB (a) without and (b) with a ceramic resonator at the optimal position for a rotary biscuit; the X-axis represents the microwave frequency in GHz, and the Y-axis represents S11 in dB.

4.3.3. Microwave baking of laminated biscuits

The dough sample (a round disc with a 3 cm diameter and 2 mm thickness) was placed at the optimal position in the microwave cavity. The initial mass of the laminated dough sample was approximately 3.5 g. Inner temperatures of a laminated biscuit in the microwave cavity, with an incident microwave power of 30 W, were measured using four optical fibres (Figure 41). Results show that after 15 min of baking, the average temperature of the biscuit reached about 130°C. ANOVA results indicated that significant differences were observed across all baking intervals. The temperature variation across different probe locations within the biscuit confirms the non-uniformity of microwave baking, which is the main challenge in microwave heating (Ahmed & Ramaswamy, 2007; Ramaswamy & Pillet-Will, 1992; Sumnu et al., 2005; Vadivambal, 2010).

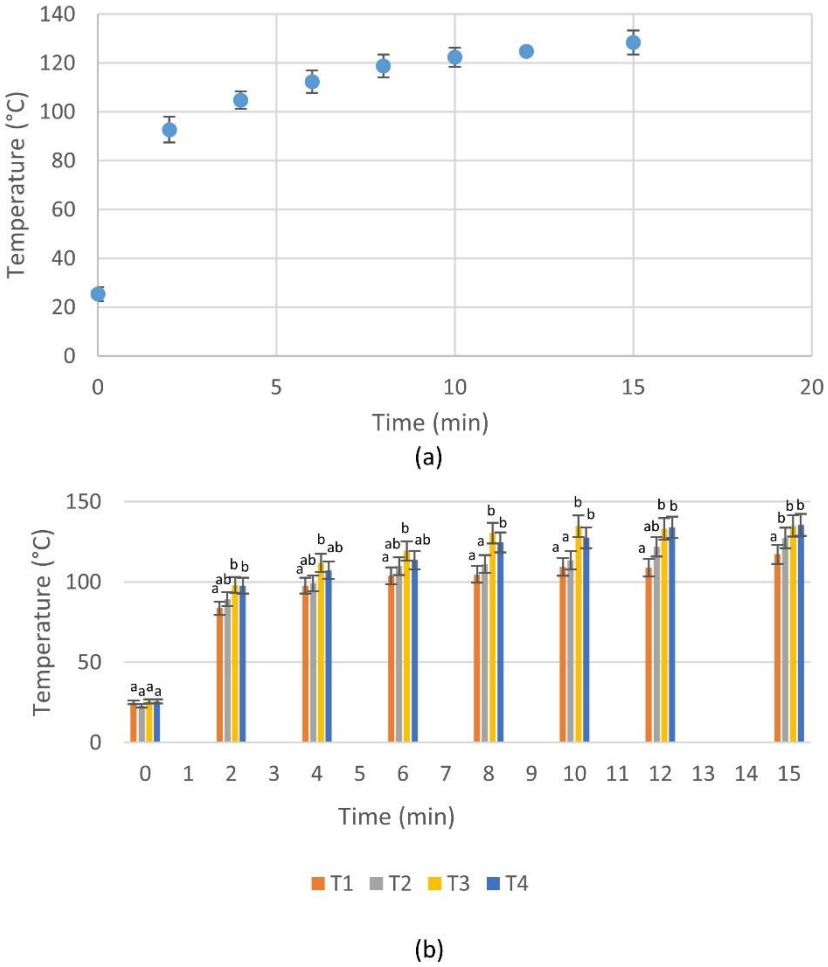

Figure 4.11. (a) Temperature development of a laminated biscuit in the microwave cavity at a power of 30 W (b) temperature registered at four spots of a laminated biscuit using optical fibres; bars with the same letter at each baking time are not significantly different (95% confidence interval).

The obtained results are in accordance with the findings of Ramaswamy and Pillet-Will (1992). They studied temperature distribution in microwave-heated food products (spaghetti with meat sauce, rice with salmon, and white sauce) at 2.45 GHz using a domestic microwave without a turntable. Temperatures were measured after heating using thermocouples. They found large temperature differences — up to 65°C between hot and cold spots — indicating strong heating inhomogeneity. The similar findings were observed by Manickavasagan et al. (2009). In this study, microwave heating of ready-to-eat chicken pie in three domestic ovens found non-uniform heating patterns with hot and cold spots both on the surface and inside. Surface temperatures were higher than internal ones at all positions. Temperature non-uniformity ranged from 31.6 to 130.5°C on the surface and 10.7 to 76.1°C internally. Using a turntable reduced surface non-uniformity, but the pie's location in the cavity had little effect on internal temperature uniformity. The maximum temperature difference in a microwave-baked laminated biscuit, after 15 min of baking, was 18.27°C.

The mass loss percentage of a laminated biscuit was measured over different baking durations (Figure 4.12). It can be observed that up to 10 min, the mass decreased at a higher rate (steeper slope), but after that, the rate of mass loss slowed down. This can be explained by the reduced water content due to evaporation; as a result, microwave heating becomes slow. At the end of baking, the laminated biscuit had a water content of 6.40% ± 0.42 (d.b.).

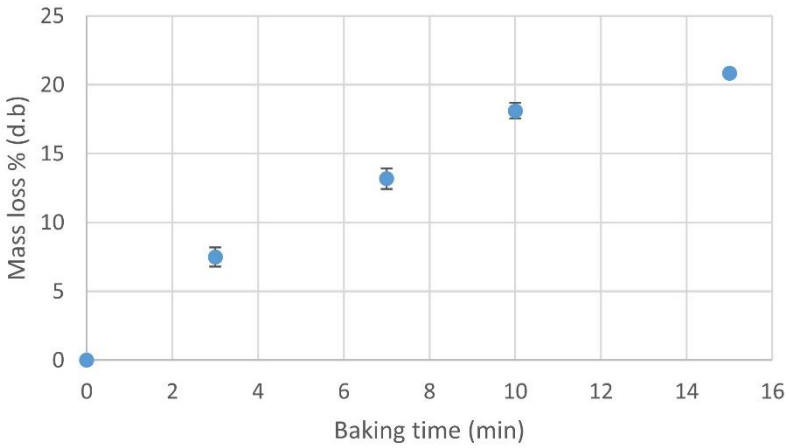

Figure 4.12. Mass loss of a microwave-baked laminated biscuit at a power of 30 W during 15 min baking.

Since the microwave-baked biscuits were pale (Figure 4.13), they were placed in an infrared oven equipped with five halogen lamps for 30 s to improve the crust colour and, if possible, further reduce the water content, as a final water content of below 5% (w.b.) is required.



Figure 4.13. (a) Microwave-baked laminated biscuit and (b) combined microwave-infrared baked laminated biscuit.

The infrared baking did not change the water content, but led to more appealing biscuits for consumers (Figure 4.13). As it can be observed, the crust colour was not homogeneous; however the colourimetric measurements are suggested for detailed results.

4.3.4. Microwave baking of rotary biscuits

Similar to the laminated biscuits, the optimal baking position for rotary biscuits was identified using the VNA connected to the microwave cavity. Rotary biscuits were baked one at a time with an incident power of 30 W for 10 min, as longer baking durations resulted in burning. The internal temperature of the biscuits was measured using four optical fibres (Figure 4.14). Similar to laminated biscuits, ANOVA

showed significant differences across all baking intervals. The internal temperature of the rotary biscuit reached approximately 100°C at the end of 10 min of baking.

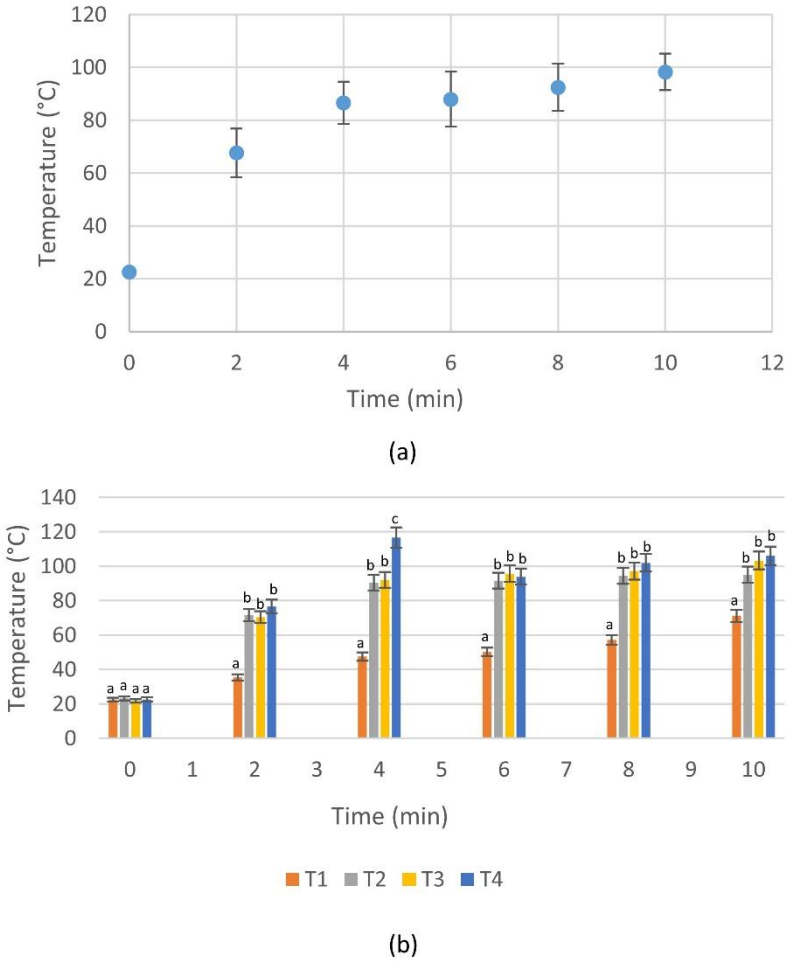

Figure 4.14. (a) Temperature development of a rotary biscuit in the microwave cavity at a power of 30 W (b) temperature registered at four spots of a rotary biscuit using optical fibres; bars with the same letter at each baking time are not significantly different (95% confidence interval).

The evolution of mass loss in the rotary biscuits is demonstrated below (Figure 4.15):

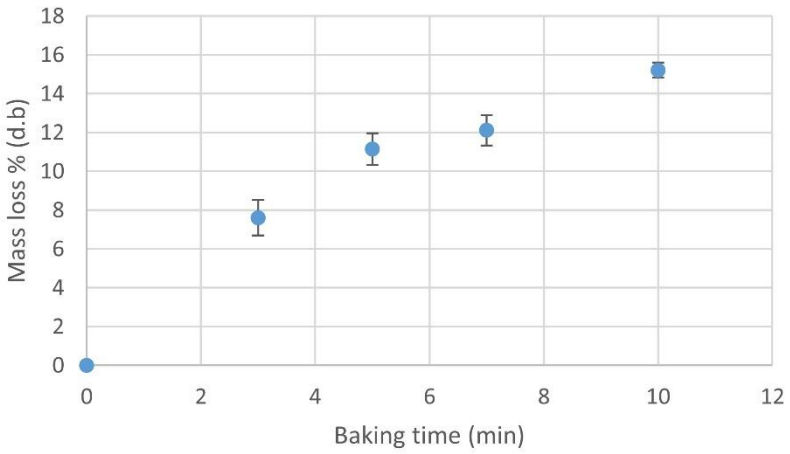

Figure 4.15. Mass loss of a microwave-baked rotary biscuit at a power of 30 W during 10 min baking.

The average water content of the microwave-baked rotary biscuits was 6.37% ± 0.13 (d.b.) at the end of baking. Rotary biscuits were placed in an infrared oven for 30 s colour enhancement (Figure 4.16).

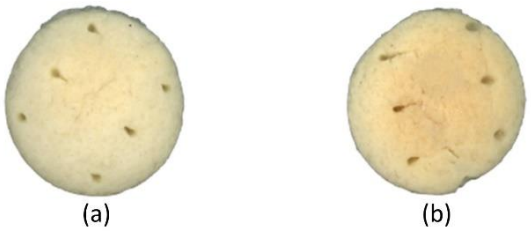

Figure 4.16. (a) Microwave-baked rotary biscuit and (b) combined microwave-infrared baked rotary biscuit.

Infrared baking did not affect the water content of the rotary biscuits; however, it improved the crust colour.

4.3.5. Microwave energy consumption

4.3.5.1. Efficiency of the microwave oven

Microwave heating of water, in terms of the time required to reach a temperature of approximately 50°C, was measured by placing different volumes of water in the microwave cavity, without a resonator, and applying an incident microwave power of 40 W (Figure 4.17). The final temperature of the water was measured at the end of microwave treatment by mixing the liquid in the container.

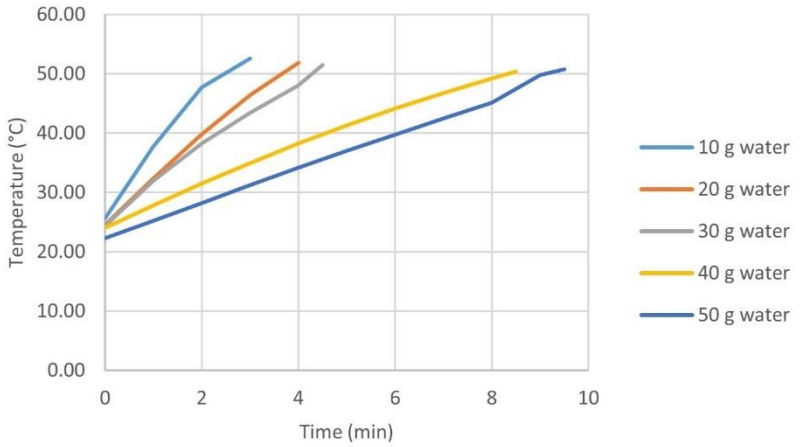

Figure 4.17. Temperature evolution of water in the microwave cavity at an incident microwave power of 40 W.

It was observed that, at a constant power of 40 W, increasing the quantity of water in the microwave cavity resulted in a longer time to reach a temperature of 50°C. Considering the C_p of water as 4.186 J /g °C, the P_{eff} absorbed within the water in the cavity was calculated as follows (Table 4.16):

Table 4.16. Microwave efficiency (%) for heating liquid water at an incident microwave power of 40 W.

Quantity of water in the microwave cavity (g)	P_{eff} (W)	P_{ref} (W)	Efficiency (%)	Mass loss (%)
10	5.91	0.00	14.78	0.40
20	9.03	0.00	22.58	0.20
30	11.89	0.00	29.72	0.23
40	10.09	4.00	28.03	0.27
50	11.00	5.00	31.43	0.30

The absorbed energy by biscuits (Q) was then determined using the formula found in Section 3.3.1.4 of the Materials and Methods. In order to calculate the efficiency of the microwave oven, the temperature changes profile, from 20°C to around 60°C was considered and the absorbed energy was calculated. After 1 min of microwave baking, the laminated and rotary biscuits reached a temperature of approximately 60°C. The C_p of laminated and rotary biscuits obtained from DSC, are 2.40 and 2.39 J/(g °C), respectively.

The microwave efficiency tests with water conducted using 10 g of water was the minimum sample size needed for accurate optical fibre temperature measurements. This amount was required to

guarantee that the temperature probes were properly submerged and to get consistent representative temperature measurements. Nevertheless, the volume of a single biscuit sample is smaller than the corresponding water volume (~10 mL). Because of this, the material and the microwave field interact differently. In addition, they are two different matrices. While smaller biscuit pieces are exposed to a less uniform field distribution, larger samples occupy a larger portion of the cavity and intercept more of the electromagnetic field. Due to these experimental limitations, the microwave power efficiency was determined using the energy absorbed by each biscuit when the temperature changed from 20°C to around 60°C. Despite the fact that the absolute efficiency values cannot be directly applied to the biscuit dough because of variations in sample volume and dielectric characteristics, this method offers an estimate of the oven's overall performance.

4.3.5.2. Baking energy

The specific energy of the biscuit dough (SE) was calculated following the method explained in Section 3.3.1.4 of the Materials and Methods. Assuming an efficiency of 18.60% and 17.52% for the SAIREM microwave oven for laminated and rotary biscuits, respectively — meaning that only 18.60% and 17.52% of the input energy is effectively used for baking biscuits — the actual absorbed energy values are as follows (Table 4.17):

Table 4.17. Energy consumption of the SAIREM microwave oven for baking biscuits.

Type of dough	Specific energy (SE, J/g dough)	Absorbed energy (J/g dough)
Laminated	516.68	96.10
Rotary	615.40	107.82

The results revealed high energy consumption by the microwave oven during biscuit baking, primarily due to its low efficiency. These findings indicate the need for a more efficient microwave oven design to reduce energy consumption. The higher energy consumption observed for laminated biscuits compared to rotary biscuits may be linked to their longer baking duration.

4.3.6. Conclusion

In microwave baking, a ceramic resonator can improve energy absorption by the biscuits within the microwave cavity. However, the temperature distribution during microwave baking was not homogeneous. The temperature profiles recorded by optical fibres showed a more homogeneous heating of laminated biscuits compared to rotary biscuits. During the process, laminated and rotary

biscuits reached temperatures of 130°C and 100°C, respectively, at an applied power of 30 W, suggesting a lower level of AA formation. However, the final water content was above 5% (w.b.), which is undesirable. The biscuits also appeared pale in colour.

To improve product quality, a secondary baking step using infrared baking was applied. While this secondary baking improved the crust colour, the colour development remained non-uniform and did not reduce the water content. For a more accurate comparison, colour measurements of biscuits produced using conventional, microwave, and combined microwave-infrared baking — using samples of the same shape and size — are recommended.

4.4. Article 2: Impact of biscuit formulation and baking on acrylamide formation

In this part, the results of AA analysis for conventional, microwave, and combined microwave-conventionally baked laminated biscuits (LB-ABC-CB, LB-ABC-MW, LB-ABC-MW-CB), as well as for conventional, microwave, and combined microwave-conventionally baked rotary biscuits (RB-ABC-CB, RB-ABC-MW, RB-ABC-MW-CB), are presented in the form of an article. This article is intended for submission to the *Food Research International* journal.

Reducing acrylamide levels in biscuits: the interest of mixing under CO_2 to reduce ammonium bicarbonate, and the application of microwave baking

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Abstract

This study investigated two strategies to reduce acrylamide (AA) formation in biscuits: microwave baking and dough mixing under CO_2 pressure to reduce or eliminate the use of ammonium bicarbonate (ABC) as a leavening agent. Both laminated and rotary biscuits were studied to evaluate the impact of these processing methods on their quality. The biscuits were baked in a conveyor microwave oven, using specific energy levels of 1.41 and 1.18 MJ/kg for laminated and rotary biscuits, respectively. Some microwave-baked biscuits underwent additional conventional baking to complete the process, particularly to achieve final drying and colouring. Biscuits baked in a convetional oven served as the control. AA content was measured using LC-MS/MS, and further analyses included dough pH, water content, colour, and texture of the baked biscuits. The suppression of ABC in laminated biscuit dough, and over-pressure CO_2 mixing as an alternative leavening method resulted in a significantly lower AA content compared to the control (555 vs. 201 ng/g dry biscuit). AA levels in microwave-baked laminated and rotary biscuits also decreased noticeably, from 555 to 71 ng/g dry biscuit, and from 128 to 24 ng/g dry biscuit, respectively. However, the combination of microwave and conventional baking led to higher AA levels than microwave baking alone, due to the higher temperatures used in the final baking step. Sensory analysis showed that conventionally-baked biscuits were still the most preferred by panellists compared to combined microwave-conventionally baked biscuits. Nevertheless, biscuits

produced with a combined microwave-conventional baking present a promising alternative to fully conventionally-baked biscuits.

Keywords: Laminated biscuit; Rotary biscuit; CO_2 mixing; Microwave baking, Acrylamide

4.4.1. Introduction

Neo-formed contaminants (NFCs) are commonly found in foods, particularly those processed at high temperatures (above 120°C). These contaminants include by-products from processes like the Maillard reaction, sugar reduction, the thermal breakdown of polyphenols, and lipid oxidation. Specific compounds, such as AA, furan, furfuryl alcohol, and hydroxymethylfurfural, are consistently associated with potential cancer-causing effects. NFCs are prevalent in widely consumed foods, such as bakery products, coffee, and cocoa, which are frequently consumed worldwide (Medina-Orjuela et al., 2024).

AA is a colourless, tasteless, and water-soluble substance that reacts with iron salts, bases, acids, and oxidising agents (Tepe & Çebi, 2019). Ammonia, CO_2, CO, and nitrogen oxides are the products of AA's thermal breakdown (Kitahara et al., 2012). The International Agency for Research on Cancer (IARC) has categorised AA as a group 2A carcinogen, indicating it is probably carcinogenic to humans (International Agency for Research on Cancer & International Agency for Research on Cancer, 1994). It can affect metabolism, reproduction, normal cell division, and developmental failure. When consumed, it is transferred to various organs such as the brain, liver, breast, kidneys, and heart. During metabolism, the body converts it to glycidamide, which has the capacity to seriously harm DNA. Additionally, AA use at low doses for prolonged periods of time may affect neurotransmission and axonal efficiency (Maan et al., 2022).

Asparagine is the key amino acid involved in AA formation (Van Der Fels-Klerx et al., 2014; Yaylayan et al., 2003). For potatoes and cereals, selecting low-asparagine types is advised to lower AA formation (Loaëc et al., 2014). Through the processes of deamination and decarboxylation, asparagine can degrade thermally. However, in the presence of a carbonyl source, asparagine's ability to form AA is significantly increased. This helps explain why foods like baked goods and fried potatoes that are high in both reducing sugars and free asparagine tend to have higher levels of AA (Capuano & Fogliano, 2011).

In AA formation, reaction speed is also impacted by the physical state of sugars. Before reacting with asparagine or forming carbonyl intermediates that result in the formation of AA in dry systems, crystalline sugars must first melt. This helps explain why fructose produces more AA under comparable heating conditions in dry environments, even though it is a ketose and typically less reactive than aldoses like glucose (Van Der Fels-Klerx et al., 2014).

The main process by which AA is formed from reducing sugars and asparagine is the Maillard reaction (Alsafra et al., 2023; Capuano & Fogliano, 2011; Maan et al., 2022). The Maillard reaction forms AA in foods high in starch (Maan et al., 2022). Lipid thermal degradation has been suggested as another mechanism for the formation of AA (Figure 4.18). Acrolein and acrylic acid are produced when lipids dehydrate at high temperatures (Adebo et al., 2017). When heated with amino acids, particularly asparagine, these lipid-oxidising compounds produce AA (Ehling et al., 2005).

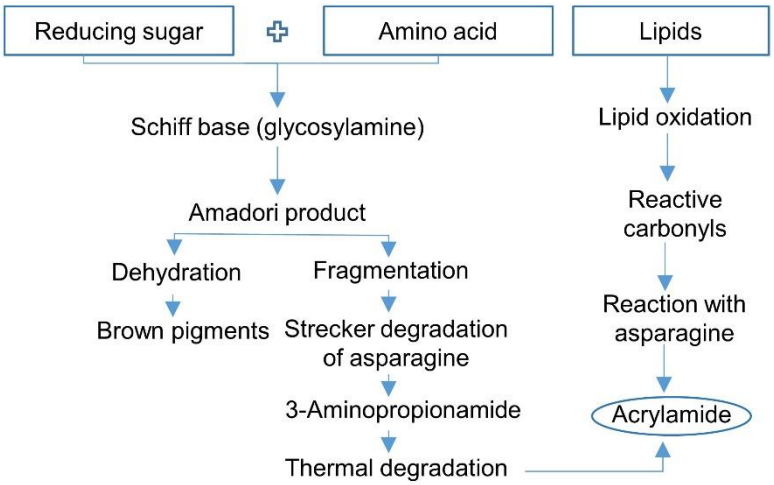

Figure 4.18.Acrylamide formation through the Maillard reaction and lipid oxidation.

Several factors during wheat cultivation(such as fertiliser use, harvesting methods, and storage conditions), as well as during the baking process (including baking method, temperature, time, dough pH, surface area, and water content), can influence the formation of AA in food (Govindaraju et al., 2024). The composition and structure of the biscuit matrix significantly affect AA formation. In a research study conducted by Anese et al. (2011), it was observed that higher water content enhanced reactant mobility, which promoted AA formation. In contrast, the fat content limited the reactant interaction. Replacing the fat phase with a monoglyceride–palm oil–water gel produced results similar to those of fat-free biscuits (Anese et al., 2011). Thermal processing methods such as baking, frying, and roasting are among the most common food processing techniques (Maan et al., 2022). Since heat triggers AA formation, several studies have investigated how variables like processing temperature and time influence AA levels (Qadeer et al., 2024; Van Der Fels-Klerx et al., 2014; Žilić et al., 2020). Van Der Fels-Klerx et al. (2014) observed that the amount of AA in biscuits was significantly impacted by both baking time and temperature. At 200°C, the concentration of AA peaked as the temperature rose. Longer baking times resulted in higher AA formation at all tested temperatures (180°C, 190°C and 200°C) with a linear increase seen at 200°C.

In a research study by Žilić et al. (2020), biscuits were prepared using wholegrain flours from eight small grain cereals and four maize genotypes, then baked at 180°C for 7, 10, and 13 min. AA levels

increased with baking time, reaching up to 861.7 µg/kg in hulless oat-based biscuits, while lower levels were observed in refined bread wheat and red maize biscuits. This study supports the use of low-asparagine flours and shorter baking times for mitigation on AA concentration by showing that both flour type (based on free asparagine content) and thermal load have a significant impact on AA formation. Qadeer et al. (2024) observed similar results in pizza, cake, and biscuits under three baking treatments with varying temperatures (150–230°C) and baking times (10–25 min). While lower temperatures with longer times sometimes significantly reduced or eliminated AA, the results showed that higher temperatures with shorter times produced the highest levels of AA. According to the findings, baking longer and at a lower temperature is a good way to reduce the production of AA in a variety of baked goods, including biscuits.

Some researchers have studied the impacts of microwave baking as an alternative method to traditional baking on the formation of AA (AL-Ansi et al., 2019; Dong et al., 2022). Al-Ansi et al. (2019) evaluated how adding black cumin and fennel seeds affected the quality of biscuits baked both conventionally and in a microwave oven. Biscuits baked with microwaves had higher antioxidant activity and lower AA levels (306.9 µg/kg) than conventional biscuits (355.2 µg/kg). The AA content decreased to 138.6 µg/kg in conventional biscuits and 97.8 µg/kg in microwave-baked biscuits upon the addition of black cumin seed. They observed that both the high antioxidant content of black cumin and fennel seeds and the lower thermal intensity of microwave baking, which restricts Maillard reaction conditions, were responsible for the decrease in AA levels. Dong et al. (2022) considered the relationship between microwave power and time and the development of thermal processing hazards (TPHs) in biscuits baked in a microwave. Methylglyoxal, 5-hydroxymethylfurfural, 3-deoxyglucosone, and AA levels rose with longer durations (2–3 min) and higher power (440–520 W). Notably, 3-deoxyglucosone was an essential precursor to the other TPHs. Methylglyoxal, 3-deoxyglucosone, AA, and 5-HMF were all reduced by 60.75%, 30.19%, 30.87%, and 61.28%, respectively, by microwave baking at 440 W for 3 min compared to conventional baking (190°C, 7 min).

Apart from the process conditions, AA formation in carbohydrate-rich foods is reduced in low pH conditions (Govindaraju et al., 2024; Khorshidian et al., 2020; Pandiselvam et al., 2024). During the Maillard reaction, the pH level is essential for the formation of AA, especially during processes like Schiff base formation, Heyns rearrangement, and Amadori compound transformation. The protonation of amino groups inhibits their interaction with carbonyl compounds, which reduces the formation of AA in acidic environments. Alkaline conditions, on the other hand, enhance the formation of AA by deprotonating amino groups, which transforms them into reactive nucleophiles that accelerate the reaction (Govindaraju et al., 2024). ABC, a common chemical leavening agent in the biscuit industry, promotes AA formation by releasing ammonia (NH_3), which raises the dough's pH and

creates an alkaline environment that accelerates the Maillard reaction (Masatcioglu et al., 2014; Palermo et al., 2016). To mitigate AA formation, one effective strategy is replacing ABC with a non-ammonia-releasing leavener. In a study by Lo Faro et al. (2022), partially substituting ABC with sodium bicarbonate (SBC), lowering the baking temperature, and applying steam treatment resulted in an 87.2% reduction in AA levels compared to control biscuits.

Researchers evaluated CO_2 gas hydrates (GH) instead of ABC as a leavening agent in black-and-white cookies. Tests were conducted with GH concentrations of 20%, 40%, and 50%. Cookies made with 50% GH had slightly more than half the volume of those made with ABC. The dough's textural qualities held up well in spite of the decreased volume and cooling effect. GH was synthesised for three hours at 0–1°C and 36–37 bar. Significantly, the amount of AA dropped from 24.8 µg/kg to approximately 18 µg/kg, suggesting an improvement in health properties (Kollemparembil et al., 2023).

Research investigating the use of CO_2 during biscuit dough mixing and microwave baking as alternatives to conventional methods—especially in relation to their influence on AA formation—remains limited.

Furthermore, suppressing ABC and the introduction of over-pressure mixing using CO_2 is expected to increase the void space in the dough, as gas bubbles serve as nucleation sites for structure development during baking. Additionally, mixing in an environment high in CO_2 may promote biscuit expansion since the dissolved CO_2 released during baking, facilitating the rising process in the oven.

This study aims to evaluate the effect of removing ABC, a major precursor to AA formation, on AA reduction. To support this objective, the dough was mixed under CO_2 gas intending to dissolve the CO_2 in the dough to help maintain a certain level of expansion during baking, thereby preserving the aerated structure of the biscuits. Additionally, microwave baking was considered as an alternative to conventional baking to lower the temperature reached during baking, since AA formation is associated with the temperature during baking, and its impact on AA concentration was examined in two types of biscuits: laminated and rotary moulded biscuits. Besides properties such as colour, texture, and others, the acceptability of these biscuits was assessed through sensory analysis tests.

4.4.2. Materials and Methods

4.4.2.1. Materials

4.4.2.1.1. Biscuit ingredients

Biscuit doughs were prepared using type T55 wheat flour (Enilia, France), characterised by a water content of 14.34% (w.b.), protein content of 9.60%, damaged starch level of 14.3 UCD, and 0.58% ash.

This flour was a biscuit flour made from soft wheat. Additional ingredients included 82% fat butter (Président, France), icing sugar and powdered sugar (Béghin Say, France), salt, water, glucose syrup (Vahiné, France), 1.5% fat skim milk powder (MATINES, France), ABC, and SBC (both from Brenntag, Germany), and SAPP 10 (Budenheim, Germany). SAPP 10 was chosen as a slow-acting leavening agent, releasing only 10% of the CO_2 from SBC during dough mixing.

4.4.2.1.2. Chemicals for Acrylamide Analysis

AA powder (≥ 99%) was obtained from Sigma-Aldrich (Germany), and acrylamide-2,3,3-d_3 powder (> 98%) was obtained from C/D/N Isotopes Inc. (Canada). Water and methanol (HPLC grade) were supplied by Chem-Lab NV (Belgium). Carrez I (potassium hexacyanoferrate (II) trihydrate) and Carrez II (Zinc Sulfate Heptahydrate) were purchased from Fluka Biochemika (Switzerland). Oasis MCX cartridges (1 cc) (Waters™, Ireland) were used for AA extract purification.

4.4.2.2. Biscuit Preparation

4.4.2.2.1. Biscuits Formulation

Seven types of biscuits were prepared with three baking methods: conventional baking, microwave baking, and combined microwave-conventional baking:

- Conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (LB-ABC-CB) as a control for laminated biscuits
- Conventionally-baked laminated biscuit free from ABC, containing SBC and SAPP 10 with CO_2 over-pressure mixing (LB-CO2-CB)
- Microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10 (LB-ABC-MW)
- Combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10 (LB-ABC-MW-CB)
- Conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (RB-ABC-CB) as a control for rotary biscuits
- Microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10 (RB-ABC-MW)
- Combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10 (RB-ABC-MW-CB) (Figure 4.19).



Figure 4.19. Biscuits with different formulations baked with different baking methods; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10).

The biscuit formulation is detailed as follows (Table 4.18):

Table 4.18. Formulation of biscuits.

	Mass of ingredients (g) per 100 g of flour (w.b)		
Ingredients	LB-ABC	LB-CO2	RB-ABC
Flour	100	100	100
Butter	5.53	5.53	27.75
Icing sugar	31.85	31.85	-
Powder sugar			
Water	20.77	20.77	35.24
ABC	1.00	-	0.44
SBC	0.64	0.64	0.44
SAPP 10	0.40	0.40	0.46
Milk powder	-	-	1.76
Salt	0.94	0.94	0.44
Glucose syrup	2.23	2.23	-
Total	**163.36**	**162.36**	**175.27**
Dough hydration (%)	**22.44**	**22.65**	**15.77**

(LB-ABC: laminated biscuit containing ABC, SBC, and SAPP 10; LB-CO2: CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; RB-ABC: rotary biscuit containing ABC, SBC, and SAPP 10).

4.4.2.2.2. Dough Mixing, Shaping, and Baking

The preparation methods for laminated and rotary biscuits are described in detail in Appendix A.

4.4.2.3. Dough and Biscuit Quality

4.4.2.3.1. Dough pH

The pH of biscuit dough was measured at 25°C using a solid electrode connected to a pH meter WTW 3110 (Xylem Analytics, Germany) until stabilisation.

4.4.2.3.2. Water Content

The water content of the biscuits was determined according to AACC Method 44-15.02. 3 g (w.b.) of ground biscuit sample was placed in an aluminium dish and dried in an oven at 105°C for 24 hours. Following drying, the sample was cooled in a desiccator for 10 min prior to weighing. All measurements were conducted in triplicate to ensure accuracy.

4.4.2.3.3. Colour

The surface colour of the biscuits was assessed using a MetaVue colourimeter (X-rite, US), following the CIELAB colour space system. This system quantifies colour using three parameters: L* (lightness), ranging from 0 (black) to 100 (white); a*, indicating the red–green axis (negative values denote green, positive values indicate red); and b*, representing the blue–yellow axis (negative values for blue, positive values for yellow). For each type of biscuit, three individual biscuits were randomly selected. On each biscuit, colour readings were taken at five distinct surface points**,** and the mean value was calculated. The instrument was routinely calibrated to ensure measurement accuracy throughout the analysis. The Browning index (BI) was then calculated based on the CIE values of L* a* b* according to the following equations (Masciola et al., 2025):

$$BI = \frac{(-0.31) \cdot 100}{0.17} \qquad \text{Eq. 4.7}$$

Where X is:

$$X = \frac{a^* + 1.75 \cdot L^*}{5.645 \cdot L^* + a^* - 3.012 \cdot b^*} \qquad \text{Eq. 4.8}$$

4.4.2.3.4. Texture

The texture (hardness) of biscuits was measured using a texture analyser (AMETEK-TA, US) with a 1 kN load. The three-point bending test was applied to measure the hardness. The measurement pre-test speed of 1 mm/s, test speed of 5 mm/s, and a distance of 10 mm were applied. The span between the

supports was 35 mm. Textural attributes were measured in five samples. The peak breaking force (N) was determined by analysing the force-displacement plots.

4.4.2.4. Acrylamide Analysis

4.4.2.4.1. Sample Preparation

AA analysis of the biscuits was performed after a triple-stage extraction as described by Žilić et al. (2024) with some modifications. Seven biscuits were finely ground in a grinder prior to extraction. A 10 mM formic acid solution containing 20 ng/mL of d_3-acrylamide was prepared as the extraction solvent. In the first extraction step, 0.5 mL Carrez I, 0.5 mL Carrez II, and 9 mL of the extraction solution were added to 1 g of finely ground sample and vortexed for 3 min. The supernatant was then removed and collected in a tube after centrifugation for 10 min at 8000 rpm at 4°C. In the second extraction step, 5 mL of extraction solution was added to the pellet, vortexed for 3 min, and then centrifuged at 8000 rpm at 4°C for 10 min to separate the supernatant, which was collected in a tube. The third extraction followed the same stages as the second. All supernatants were combined in a tube, sealed, and stored at 4°C until analysis.

4.4.2.4.2. Extract Purification by Solid Phase Extraction (SPE)

The collected supernatant was then centrifuged at 8000 rpm for 20 min at 4°C. The Oasis MCX cartridge (1 cc) was prepared by adding 1 mL of methanol (HPLC grade), eluting at a rate of 1 drop/s, followed by the addition of 1 mL of water (HPLC grade), which was removed at a rate of 1 drop/s. Then, the supernatant of the centrifuged extract was passed through a preconditioned Oasis MCX cartridge. The first 8 drops of the extract were discarded, and the remaining drops were collected into an Eppendorf. The collected extract was filtered through a 0.22 µm nylon filter and transferred into vials prior to LC–MS/MS analysis.

4.4.2.4.3. LC-MS/MS Analysis of Acrylamide

The clear extracts transferred into vials were analysed using an Agilent 1260 LC system coupled to an Agilent 6420 Triple Quadrupole detector (Agilent Technologies, US). Chromatographic separation was performed on a Zorbax XDB C18 column (2.1 × 150 mm, 3.5 µm; Agilent, Waltham, MA, US). The mobile phase consisted of 10 mM formic acid in water, delivered at a flow rate of 0.3 mL/min, and the column temperature was maintained at 25°C. The injection volume was 5 µL. The electrospray ionisation (ESI) source was operated with a gas temperature of 350°C, capillary voltage of 2000 V, gas flow of 11 L/min, and nebuliser pressure of 60 psi. Data acquisition was performed in multiple reaction monitoring (MRM) mode. The precursor ion $[M + H]^+$ at m/z 72 was subjected to fragmentation, and the resulting product ions at m/z 55 (quantifier, collision energy of 9 V), m/z 44 (qualifier, CE 12 V), and m/z 27

(qualifier, CE 24 V) were monitored. Deuterated AA (d_3-acrylamide) was used as the internal standard, with a precursor ion at m/z 75 and a product ion at m/z 58. The dwell time for all MRM transitions was set to 0.2 s. A calibration curve spanning a concentration range of 1–100 ng/mL was used for the quantification of AA in the samples. The limits of detection (LOD) and quantification (LOQ) were calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S), according to the LOD = 3.3 . (SD/S) and LOQ = 10 . (SD/S). The LOD and LOQ for AA were 3 ng/g and 10 ng/g, respectively. Two replicate measurements were performed for each sample, and the results were expressed as ng/g of sample (d.b.). The LC–MS/MS system, data acquisition, and processing were controlled using MassHunter Workstation Software (Version B.06.00, Agilent Technologies).

4.4.2.5. Sensory Evaluation

The sensory tests were conducted in accordance with the Declaration of Helsinki and with the approval of Inserm's ethical evaluation committee/Institutional Review Board (CEEI/IRB00003888). Panellists were recruited voluntarily from Oniris school, so the panel consisted of Oniris students and staff. Participants were informed about the study's purpose through an information sheet. Participants with known allergies or intolerances to the biscuit ingredients were excluded from the study, and those who agreed to participate signed a consent form after reading it. 69 panellists participated. The panel included 61% female and 39% male participants, indicating a higher proportion of females. Most of the panellists (48%) were in the 18–25 age group. This was followed by 14% aged 31–40, 12% aged 26–30, and two equal groups of 10% each in the 41–50 and 51–60 age ranges. A smaller 6% of participants were over 60. Overall, this profile shows a mainly young panel, with nearly half under 25. The tests took place in the sensory analysis room at Oniris, which complies with the NF EN ISO 8589 (2010) standard. The room is equipped with 30 individual booths, with controlled temperature and lighting conditions.

LB-ABC-CB, LB-CO2-CB, LB-ABC-MW-CB, RB-ABC-CB, and RB-ABC-MW-CB biscuits were included in the sensory analysis. However, LB-ABC-MW and RB-ABC-MW biscuits were excluded due to an insufficient number of samples. Conventionally-baked biscuits were prepared and baked 3 days in advance for organisational reasons. They were stored in sealed bags and kept in a dark place at ambient temperature. The samples were presented in identical cardboard boxes, each containing one biscuit from a specific recipe and coded with a three-digit random number. At the beginning of the session, each panellist received a tablet with a questionnaire developed using FIZZ software (BIOSYSTEMES, France). Panellists were also provided with a tray containing the biscuit samples and a cup of water (Evian, France). The sequence in which the biscuits were evaluated was randomised and balanced

across panellists using FIZZ software to minimise order effects. Panellists were instructed to rinse their mouths between each sample.

Overall liking was evaluated using a 9-point hedonic scale, where a rating of 1 indicated "extremely dislike," 5 represented "neither like nor dislike," and 9 signified "extremely like." For the CATA test, the panellists were given a list of 40 sensory attributes (Figure 4.20) and instructed to mark all the attributes they perceived in the product being assessed.

Yellow interior	Cereal flavour/ odour	Crispy
White interior	Caramel flavour/ odour	Grainy
Thin	Salty	Sandy
Thick	Aftertaste	Brittle
Presence of spots	Sweet	
Smooth surface	Cake batter flavour/ odour	
Golden colour	Baking powder flavour/ odour	
Brown colour	Flour flavour/ odour	
Homogeneous appearance	Acid	
Heterogeneous appearance	Hard	
Bumpy surface	Doughy	
Brillant	Dry	
Matte	Melt-in-the-mouth	
Buttery flavour/odour	Absorbs saliva	
Vanilla flavour/odour	Crumbly	
Toasted flavour/ odour	Crunchy	
Fatty sensation	Soft	
Milky flavour/odour	Floury	

Figure 4.20. List of CATA sensory analysis attributes.

4.4.2.6. Statistical Analysis

To investigate the effects of biscuit formulation baked conventionally, using a microwave, or combined microwave-conventional on dependent variables like AA content and colour, a one-way ANOVA (analysis of variance) was applied with STATGRAPHICS 19 software (Statgraphics Technologies, US). Fishers' Least Significant Difference (LSD) post hoc analysis was performed to identify significant differences at the 95% confidence level. PCA was used to simplify the data and find patterns while preserving a significant amount of information as multiple variables were being examined. Additionally, it facilitates the discovery of variable correlations. XLSTAT (2022.3.1.1333, Addinsoft, France) was used to perform PCA. The result of the CATA test was analysed using XLSTAT (2022.3.1.1333, Addinsoft, France) with Cochran's Q test. The goal was to identify significant differences between product attributes based on panellists' responses, without excluding non-significant attributes.

4.4.3. Results and Discussion

4.4.3.1. Quality of Dough and Baked Biscuits

4.4.3.1.1. Dough pH

Results showed that except for LB-CO2-CB, which has the lowest dough pH and differs significantly from other biscuit formulations, laminated and rotary biscuits had nearly identical ingredients and therefore did not differ significantly in pH (Figure 4.21). This could be because of the acidic condition of the dough due to the removal of ABC and over-pressure mixing with CO_2. Since AA formation in food decreases at lower pH values, and the pH level has a significant impact on the mechanisms underlying the Maillard reaction (Govindaraju et al., 2024; Khorshidian et al., 2020), biscuits free from ABC (LB-CO2-CB) may have less AA than those with ABC (LB-ABC-CB, control), LB-ABC-MW, and LB-ABC-MW-CB). However, the impact of the baking method must be taken into account.

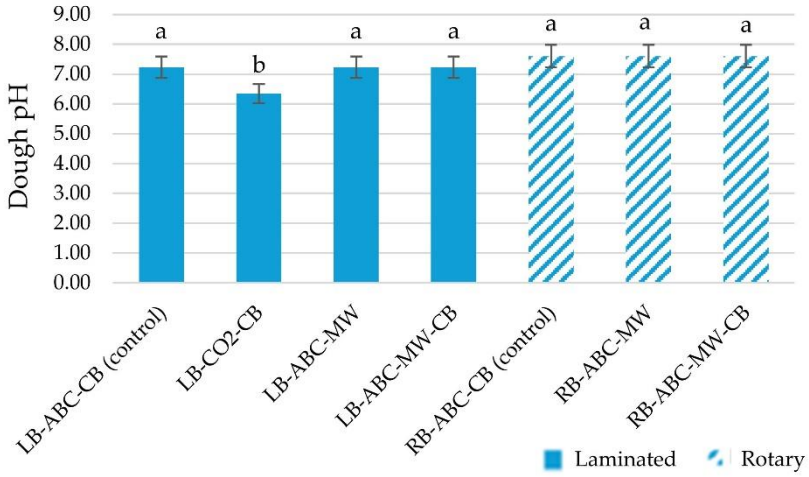

Figure 4.21. pH of biscuit dough for different formulations; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval).

4.4.3.1.2. Water Content

With the initial dough hydration of ~22% (w.b.) for laminated biscuits and ~16% (w.b.) for rotary biscuits, a one-way ANOVA results showed a significant variation in the water content of the biscuits ($p < 0.001$) (Figure 4.22), indicating that the final water content of biscuits is largely determined by the baking method. The results revealed that the RB-ABC-MW was significantly different from all other biscuits and had the highest mean water content. Although LB-ABC-MW placed second, it also

maintained a comparatively high water content, indicating that microwave baking was less successful than traditional baking at reducing mass loss. This is consistent with research on microwave-baked biscuits by Al-Ansi et al. (2019). The heat and mass transfer mechanisms of conventional and microwave baking methods help to explain this. The food in a convection oven is heated from the outside in, while this heat and mass transfer may happen in the opposite direction due to volumetric heating during microwave processing (Wilson et al., 2002), which causes the water content of the biscuits' inner and outer portions to differ noticeably (Marion et al., 2019). It was observed that microwave-baked biscuits had higher water content compared to conventional and combined microwave-conventionally baked biscuits. This finding is in accordance with a previous research work on microwave-baked biscuits (AL-Ansi et al., 2019).

Despite different formulations and initial dough hydration, there was no discernible difference between RB-ABC-MW-CB and LB-ABC-MW-CB, suggesting similar water contents. The least amount of water was present in LB-CO2-CB and RB-ABC-CB. These findings support the notion that changes in baking time and temperature can have a substantial impact on baked goods' water content (Ureta et al., 2019; Wilson et al., 2002). In the case of microwave baking, the higher power will lead to less water content in biscuits (Dong et al., 2022).

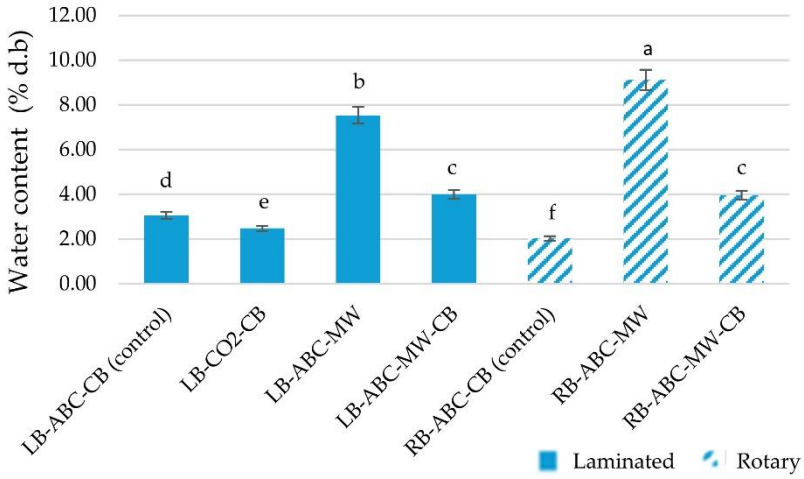

Figure 4.22. pH of biscuit dough for different formulations; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval).

4.4.3.1.3. Biscuit Colour

According to the colour analysis results (Table 4.19), the L* value in LB-ABC-CB (control) biscuit stayed at the lowest level. In comparison to the controls, LB-ABC-CB, and RB-ABC-CB, conventional and combined microwave-conventionally baked biscuits were lighter. Al-Ansi et al. (2019) also found that microwave-baked biscuits are lighter than conventionally-baked biscuits. Compared to microwave-baked biscuits, the darker colour of conventionally-baked biscuits is expected due to the higher temperature and longer baking time. This intensified heating promotes non-enzymatic browning reactions, particularly Maillard reaction, which is primarily responsible for both the development of dark colour and flavour of baked goods (Park et al., 2015; Purlis, 2010). Generally speaking, non-enzymatic browning reactions start at high temperatures, and high temperatures cannot usually be reached by microwave heating alone (Sumnu et al., 2005). LB-ABC-CB had the highest a* value, followed by LB-CO2-CB, and RB-ABC-CB. Microwave-baked biscuits (LB-ABC-MW and RB-ABC-MW) had the lowest a* value, and combined microwave-conventionally baked biscuits (LB-ABC-MW-CB and RB-ABC-MW-CB) had the second-lowest a* values. LB-ABC-CB, as a control for laminated biscuits, did not significantly differ from RB-ABC-CB biscuit.

Table 4.19. Biscuits' colour values and colorimetric distances between controls and reformulated biscuits.

Biscuit formulation and baking method	L*	a*	b*	ΔE
LB-ABC-CB (control)	62.87 ± 4.10 ^{e}	12.69 ± 2.43 ^{a}	30.67 ± 1.35 ^{c}	0
LB-CO2-CB	67.95 ± 1.60 ^{de}	12.42 ± 0.48 ^{a}	31.88 ± 0.49 ^{c}	5.23
LB-ABC-MW	86.70 ± 0.46 ^{a}	-0.46 ± 0.31 ^{d}	22.38 ± 1.19 ^{e}	28.45
LB-ABC-MW-CB	79.38 ± 1.54 ^{bc}	6.76 ± 1.24 ^{b}	30.54 ± 1.08 ^{d}	17.54
RB-ABC-CB (control)	70.58 ± 4.10 ^{cd}	11.74 ± 2.43 ^{a}	37.64 ± 1.35 ^{a}	0
RB-ABC-MW	83.58 ± 1.40 ^{ab}	1.49 ± 0.23 ^{cd}	23.82 ± 0.70 ^{b}	21.57
RB-ABC-MW-CB	80.93 ± 1.35 ^{bc}	3.66 ± 1.41 ^{bc}	31.25 ± 2.06 ^{b}	14.6

(LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); different letters in the same column indicate significant differences (95% confidence interval).

According to predetermined thresholds, the ΔE value is commonly used to compare samples and determine whether any colour difference would be noticeable to the human eye. In particular, the colour difference is imperceptible if the $\Delta E < 0.2$. While $0.2 < \Delta E < 0.5$ indicates a very slight difference,

0.5 < ΔE < 1.5 indicates a slight but noticeable variation, 2 < ΔE < 3 indicates a barely noticeable difference, while 3 < ΔE < 6 indicates a distinctly noticeable colour change. Variations between 6 and 12 are regarded as significant (Lo Faro et al., 2022). A noticeable colour difference was observed in all biscuits; however, this difference in the LB-CO2-CB biscuit was slight.

The higher AA content in biscuits is indicated by darker colour indices (Lo Faro et al., 2022). The degree of non-enzymatic browning is directly correlated with the L* value; a darker surface and a higher degree of browning are indicated by lower L* values (Yuan et al., 2016). Similarly, a shift toward red hues, indicating increased browning intensity, suggests an increase in the a* value (Dong et al., 2022). The current findings (Figure 4.23) demonstrated that the relationship between AA content and BI differed between laminated biscuits and rotary biscuits. In general, higher BI values were associated with increased AA formation, indicating that browning reactions occurring during baking contribute to AA generation. However, clear differences were observed between biscuit types. Among the laminated biscuits, the control sample (LB-ABC-CB) exhibited the highest AA concentration (555 ng/g) and a high BI value (79.93). When microwave baking was applied (LB-ABC-MW), both BI and AA content decreased substantially (BI = 28.72; AA = 71 ng/g), indicating that this processing step strongly limited browning and AA formation. The combined microwave-conventional baking (LB-ABC-MW-CB) yielded intermediate values (BI = 53.79; AA = 299 ng/g), indicating partial restoration of browning during the final baking stage.

It is also noteworthy that the laminated biscuit sample LB-CO2-CB, which was produced with a modified formulation in which ABC was removed and replaced with CO_2 over-pressure mixing, showed a different behaviour compared with the other laminated biscuit samples. ABC is known to promote AA formation during baking due to the release of ammonia, which can enhance Maillard reactions (Lo Faro et al., 2022). Therefore, the absence of ABC in LB-CO2-CB may have contributed to changes in AA formation relative to the standard laminated biscuit formulation.

In rotary biscuits, AA levels were consistently lower than in laminated biscuits, which may be related to the lower baking temperature (220°C vs. 240–250°C) and shorter baking duration. The control sample (RB-ABC-CB) exhibited the highest BI (85.72) but a relatively moderate AA concentration (128 ng/g), indicating that browning intensity did not translate into equally high AA formation. Similar to laminated biscuits, microwave-baked rotary samples showed the lowest AA levels (RB-ABC-MW: 24 ng/g; BI = 34.09), while the combined microwave-conventional baking produced intermediate values (RB-ABC-MW-CB: 77 ng/g; BI = 50.91).

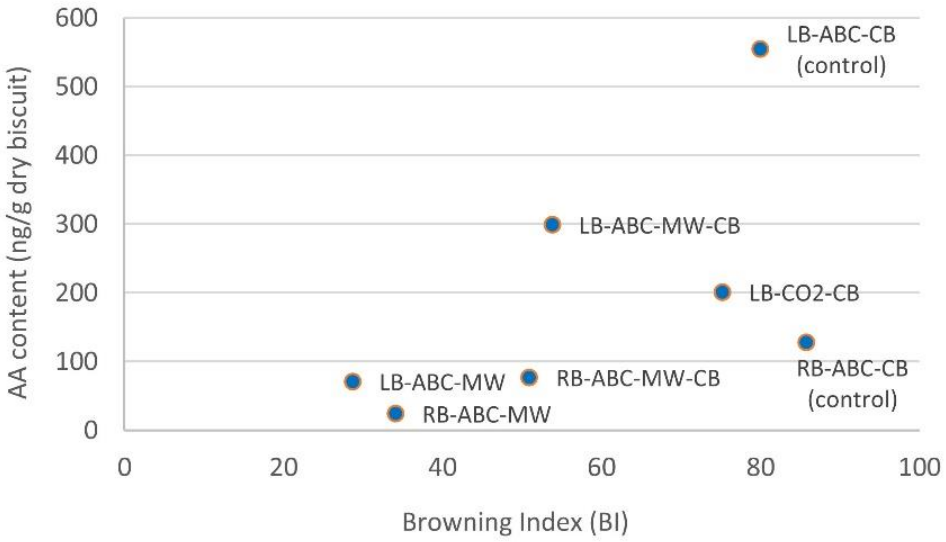

Figure 4.23. Correlation of browning index (BI) with acrylamide (AA) content of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10).

4.4.3.1.4. Biscuit Texture (hardness)

The biscuit's hardness was assessed using a three-point bending test (Table 4.20). The one-way ANOVA results showed that the baking method significantly affected the biscuits' hardness ($p < 0.05$). Notably, the LB-ABC-MW-CB biscuit outperformed all other biscuits with the highest mean breaking force (71.55 N), indicating that the combination of microwave and conventional baking methods used in this sample increased its hardness. RB-ABC-MW, on the other hand, showed the lowest breaking force (14.50 N), suggesting a more delicate texture. This can be explained by the formulation difference; rotary biscuits have more sugar and fat than laminated biscuits. Certain biscuits, like LB-CO2-CB and LB-ABC-CB, demonstrated intermediate hardness, but they could be statistically distinguished from both the best and worst-performing samples. The LB-CO2-CB biscuit was 39% harder than the laminated biscuit control (LB-ABC-CB). This is probably due to the presence of ABC in the control biscuit, which enhanced the texture.

The higher water content of microwave-baked biscuits is probably the reason why it was found that they are softer than conventionally-baked biscuits. In line with earlier research that highlights how raising the baking temperature and duration increases the biscuits' hardness in traditional baking (Verma et al., 2024). These results demonstrate the impact of the baking method on the textural

qualities of biscuits. The texture of biscuits baked in a microwave is largely determined by variations in the spatial arrangement of proteins, moisture, and starch (Leiva-Valenzuela et al., 2018).

Table 4.20. Hardness of biscuits; Hardness of biscuits.

Biscuit formulation and baking method	Hardness (N)
LB-ABC-CB (control)	27.56 ± 3.57 [c]
LB-CO2-CB	38.35 ± 1.62 [b]
LB-ABC-MW	17.85 ± 0.62 [d]
LB-ABC-MW-CB	71.55 ± 6.20 [a]
RB-ABC-CB (control)	20.73 ± 0.59 [d]
RB-ABC-MW	14.50 ± 1.98 [d]
RB-ABC-MW-CB	20.28 ± 1.58 [d]

(LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); values with the same letter are not significantly different (95% confidence interval).

4.4.3.1.5. Acrylamide Content

Baking had a statistically significant impact on AA content according to the one-way ANOVA results ($p < 0.05$) (Figure 4.24). The LB-ABC-CB (control) biscuit had the highest AA concentration and was separated from the other biscuits in a significant way. This could be explained by baking for 9 min and 30 s at a high temperature of 240°C-250°C (Suman et al., 2019).

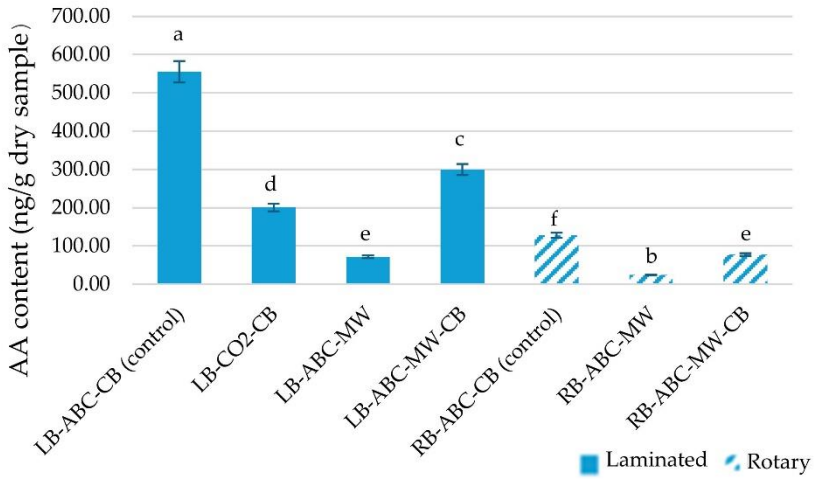

Figure 4.24. Acrylamide content of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval).

The temperature changes during conventional and microwave baking of biscuits are depicted in Figure 4.25. During conventional baking, the internal temperature reached around 140°C. However, in microwave baking, temperature changes recorded by optical fibres demonstrated that the internal temperature increased to approximately 115°C. This difference may explain the higher AA content observed in conventionally-baked biscuits. The combination of microwave and conventional baking results in biscuits with a higher AA content than microwave-baked biscuits because of the high temperature used during baking (250°C).

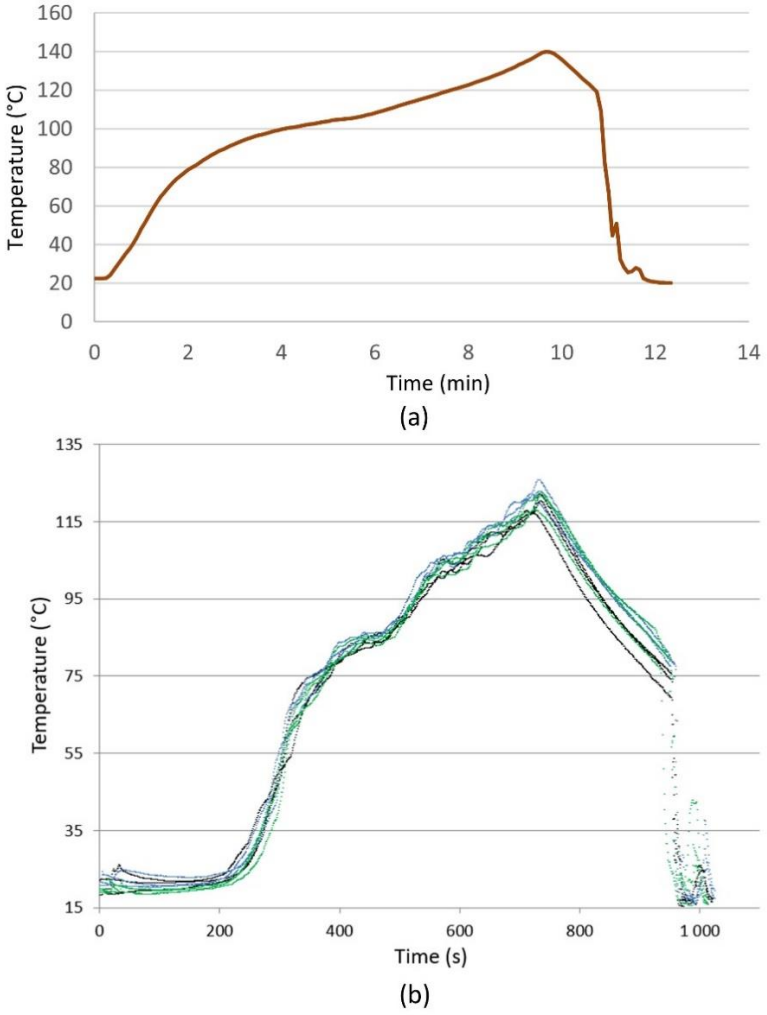

Figure 4.25. Temperature changes during (a) conventional baking and (b) microwave baking (measured for three biscuits, each at one specific location) of biscuits.

It's interesting to note that LB-ABC-MW and RB-ABC-MW-CB did not differ significantly from one another, suggesting that AA profiles were comparable even though baking methods varied. This can be explained by the lower applied microwave power (2.8 kW) used for RB-ABC-MW-CB biscuits, as well as differences in the recipe. The low baking temperature (220°C) and baking time (6 min 30 s) caused the RB-ABC-CB to have the lowest AA content, which was significantly different from all other samples. The results revealed that the baking method had a greater effect on the formation of AA in biscuits than formulation (suppressing ABC from dough), with LB-ABC-MW-CB coming in second and LB-CO2-CB in third, baked at 240°C-250°C for 10 min.

Even though the power used and baking time determine how effective microwave baking is, using the right power can lessen the amount of AA concentration in biscuits. This observation is consistent with the findings of AL-Ansi et al. (2019) and Dong et al. (2022). The benchmark levels for the presence of AA in wheat-based bread and biscuits are 50 µg/kg and 350 µg/kg, respectively (European Commission, 2017).

4.4.3.1.6. Principal Component Analysis (PCA) Analysis

According to PCA results, 77.77% of total variance was explained by F1 (60.18%) and F2 (17.59%), which means that most of the variability was captured by the first two principal components. F1 represents a*, b*, and AA in the positive side, and L* and water content in the negative side. This indicates that biscuits with high a*/b* values (more red/yellow colour), and high AA content, tend to have lower water content and lighter (higher L*). This is in accordance with previous findings observed by Lo Faro et al. (2022). F2 represents variations related to texture, where hardness contributes strongly.

The biplot (Figure 4.26) shows that rotary biscuits are all placed in the lower half of this plot, and laminated biscuits are located in the upper half. This vertical separation suggests structural and compositional differences between the two biscuit types, with laminated biscuits associated with higher hardness and browning, while rotary biscuits tend to have more water content. Additionally, a clear baking gradient is observed from right to left, with conventionally-baked biscuits on the right-associated with more intense baking characteristics such as increased AA and hardness, and microwave–baked biscuits on the left, indicating milder baking conditions.

It is observed that LB-ABC-CB is high on a*, b*, and AA, which is possibly due to more intense treatment. The LB-ABC-MW is positioned with high water content and L*, which is normal in microwave baking to have a lighter colour and more water content. RB-ABC-MW and RB-ABC-MW-CB are closer to the centre, suggesting more balanced characteristics or less extreme values. LB-ABC-CB and LB-CO2-CB are closer to AA and colour, may have undergone heat treatment leading to higher Maillard reaction.

According to this analysis, colour and AA are tightly linked — products with higher a*, b*, and lower L* tend to have more AA. Water content and colour are inversely related. Interestingly, rotary biscuits are all placed in the bottom half of this plot, and laminated biscuits are above. The microwave–baked biscuits on the left and conventionally-baked biscuits on the right.

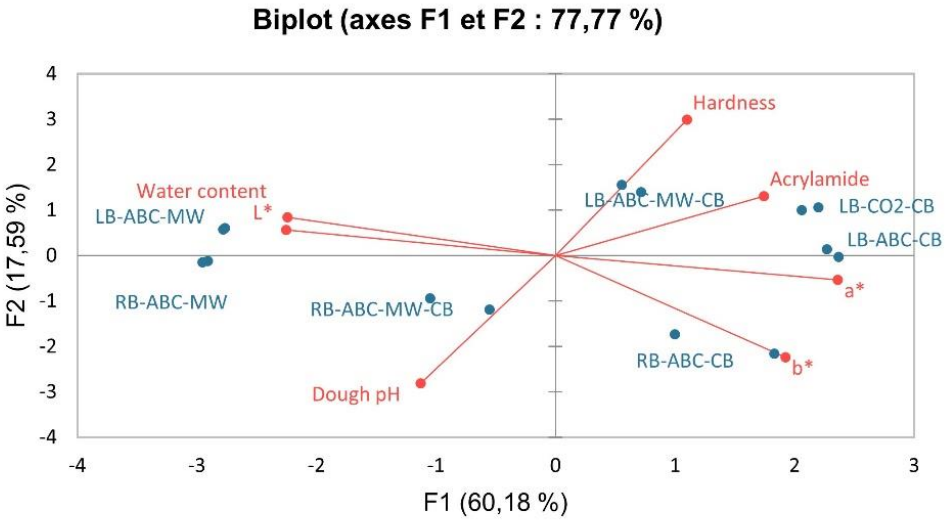

Figure 4.26. Biplot of PCA analysis of biscuits.

4.4.3.1.7. Sensory Analysis of Biscuits

4.4.3.1.7.1. Hedonic Sensory Analysis

Results revealed significant differences between the liking score of biscuits (Figure 4.27Figure 4.27). RB-ABC-CB was the most appreciated, followed by LB-ABC-CB. These samples were significantly preferred over LB-CO2-CB, LB-ABC-MW-CB, and RB-ABC-MW-CB ($p < 0.05$). The lower scores for the microwave-baked biscuits can be attributed to their shorter baking time and lower temperature, which limit browning reactions and flavour development (Sumnu et al., 2005). This suggests that conventional baking enhances sensory properties.

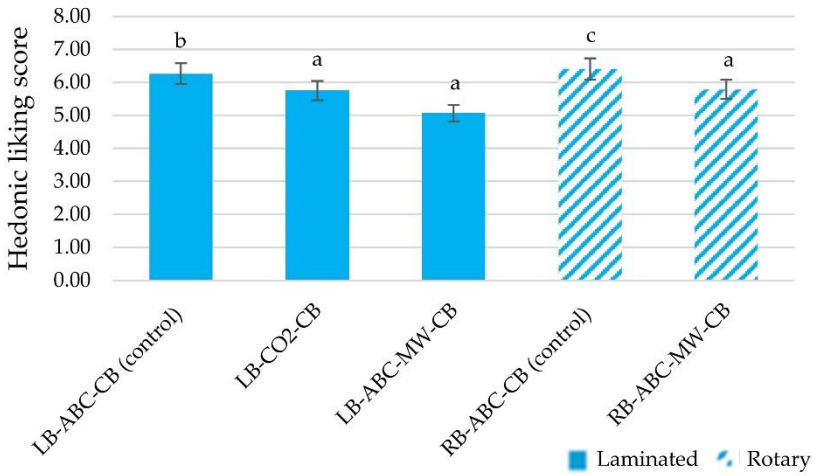

Figure 4.27. Hedonic liking score of tested biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAP; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval).

4.4.3.1.7.2. CATA Sensory Analysis

The analysis of the frequency of attributes checked by participants showed that attributes were utilised in a balanced manner, with the majority of panellists choosing between 15% and 35% of attributes. This range suggests that the list of attributes was neither too brief nor too long for the panellists. Depending on the subject, some may check less than 10% or more than 40% of the attributes.

Overall frequencies of each attribute showed that some of them were clearly dominant across the five biscuit samples. Attributes such as “crunchy” (62%), “crispy” (52%), and “dry” (50%) were among the most frequently selected. Therefore, “dryness” and “crispness” are two important sensory characteristics that set the biscuit apart. Notably, “buttery flavour/ odour” (48%) and “sweet” (46%) were regularly selected, suggesting the importance of buttery and sweet notes in the perception of these biscuits. However, some attributes like “acid” (1%), “soft” (2%), and “salty” (7%) were rarely chosen, indicating that these sensory attributes are not characteristic of the tested biscuits. It is interesting to note that some attributes related to texture, like “crumbly” (28%) and “brittle” (35%), were used moderately, indicating that they might be somewhat involved in distinguishing particular samples but are not universally accepted.

The significant results of Cochran’s Q revealed that panellists had a clear perception of variations among the five biscuits, with appearance (colour, homogeneity) serving as the primary distinguishing

factor, as well as texture (hardness, dryness, and crumbliness), and some flavour notes (sweet, roasted, milky, buttery).

The correspondence analysis biplot (Figure 4.28) clearly demonstrates distinguishing the five biscuits based on their sensory profiles. RB-ABC-CB and RB-ABC-MW-CB were associated with positive sensory attributes like sweetness, buttery and vanilla flavour, smooth surface, and crispy. However, LB-CO2-CB and LB-ABC-CB were associated with toasted flavour/ odour, brown colour, and homogeneous appearance. The weakest-performing biscuit, LB-ABC-MW-CB, was strongly associated with dry, hard, floury, grainy, floury, and aftertaste, which can be explained by the weak development of the biscuit during microwave baking. These sensory perceptions are consistent with the physicochemical measurements. For instance, the attributes “hard” and “dry”, which were frequently associated with LB-ABC-MW-CB and RB-ABC-MW-CB, align with their low water content and high hardness values, confirming the panellists’ impressions of firmer and drier textures. In contrast, RB-ABC-CB, which was described as having a “smooth surface”, “melt-in-the-mouth” texture, and “buttery flavour”, also showed higher water content and lower hardness, which explains its softer structure and greater consumer acceptance. The perception of “crunchy” and “brittle” in LB-ABC-CB correlates with its lower water content and possibly a more porous structure. These findings highlight a clear relationship between sensory attributes and the physical characteristics of the biscuits.

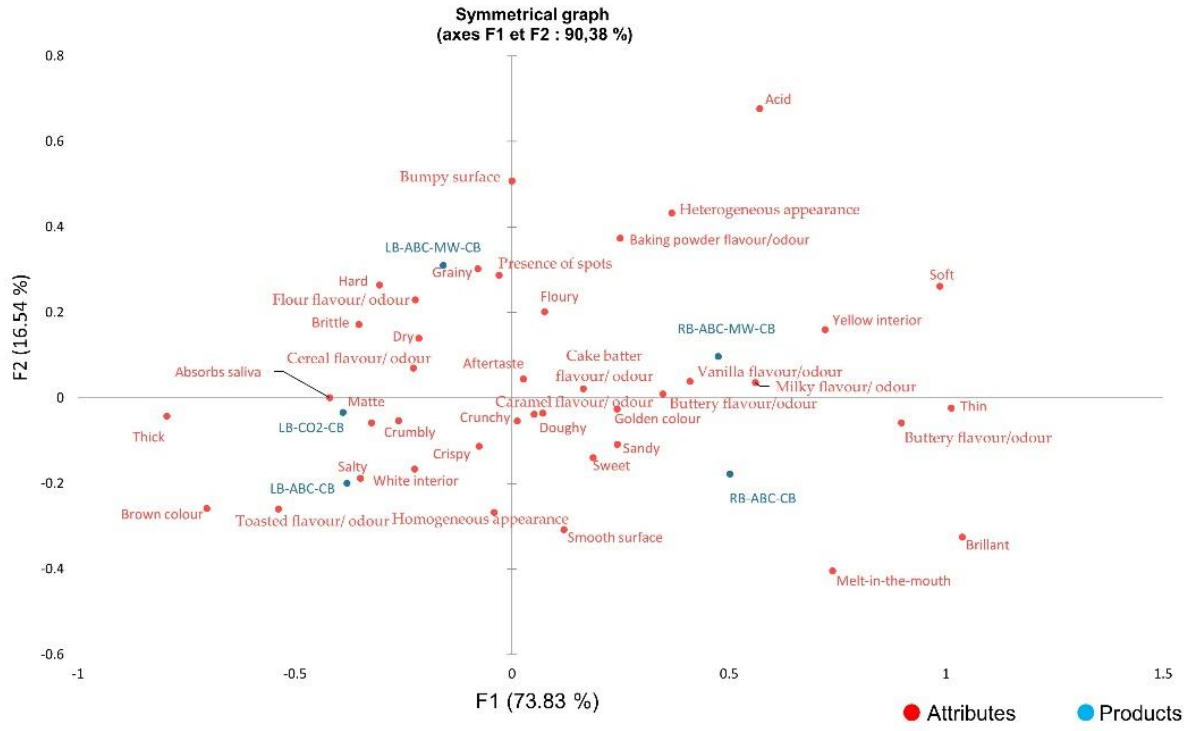

Figure 4.28. Correspondence analysis biplot of biscuits using 40 sensory attributes identified using CATA.

The analysis of CATA showed that texture and appearance were the primary discriminators. The positive consumer profile was characterised by crispy and crunchy texture, smooth surface, golden colour, and buttery and sweet flavours. The undesirable profiles were linked to dryness, hardness, grainy, and heterogeneous appearance.

4.4.4. Conclusion

The results indicate that microwave baking could be used as an alternative to traditional baking; however, since the biscuits are pale and have high water content, the second baking could improve the characteristics of the biscuits. The microwave-baked biscuits had a significantly lower AA content than the conventionally-baked biscuits. Suppression of ABC from the biscuit dough highly reduced the AA content in laminated biscuits. The positive correlation of colour and AA content was observed as well.

The analysis of CATA showed that texture and appearance were the primary discriminators for panellists and showed a positive correlation between the texture of biscuits and acceptability. LB-CO2-CB was the second preferred biscuit among laminated biscuits, indicating that it is a promising alternative to conventional leavening agents. Microwave baking of biscuits needs more consideration to improve the sensory properties of the final products.

The strategy of mixing under CO_2 gas to reduce or eliminate chemical leavening requires further development. A quick calculation shows that the chemical leavening agents in a typical biscuit recipe release a volume of gas equivalent to approximately eight times the dough volume during baking. In biscuit dough, free water and lipids are the main components capable of trapping CO_2 during mixing. However, their quantity is insufficient to dissolve such a large volume of gas. Tests using carbonated water were conducted during this project but did not result in any significant improvement, despite achieving around six times more dissolved CO_2 compared to atmospheric mixing conditions. To achieve greater expansion during baking, it will be necessary to mix under much higher pressure and to develop strategies for retaining gas nuclei within the dough.

4.4.5. Author Contributions

Conceptualisation, Alain Le-Bail, Kali Kotsiou, Maria Papageorgiou, Maryam Valizadeh, Cécile Rannou, Sébastien Curet; Methodology, Maryam Valizadeh, Kali Kotsiou, Cécile Rannou, Sébastien Curet, Alain Le-Bail; Formal analysis, Maryam Valizadeh; Data curation, Maryam Valizadeh; writing—original draft preparation, Maryam Valizadeh; writing—review and editing, Maryam Valizadeh, Alain Le-Bail, Cécile Rannou, Sébastien Curet; Supervision, Alain le-Bail, Sébastien Curet, Cécile Rannou; Project

administration and funding acquisition, Alain Le-Bail; validation, Alain Le-Bail, Sébastien Curet, Cécile Rannou, Kali Kotsiou, Maria Papageorgiou, Marie-Luce Lebriquer, Gilles Bertheau, Mathéo Bénas.

4.4.6. Acknowledgement

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4.4.7. Appendix A: Preparation of Biscuits

4.4.7.1. Dough Preparation

- **Laminated Biscuit Dough**

The laminated biscuit dough was prepared using the all-in method, where mixing begins immediately after all ingredients are added to the mixer. Prior to mixing, glucose syrup was diluted in a small volume of water, and ABC was dissolved in another portion of water. The remaining water was combined with icing sugar and salt and thoroughly mixed to prevent clumping. All ingredients were then added simultaneously into the mixer, which was equipped with a single spiral blade. A prototype batch mixer (VMI, St-Hilaire-de-Loulay, France) with an 11 L bowl volume and a maximum capacity of 5 kg of dough was used for mixing. The mixing bowl was surrounded by a water jacket to regulate the temperature of the dough during mixing. The mixer operates by rotating both the tool (agitator speed) and the satellites, while the bowl remains stationary. During the mixing process, infrared temperature sensors were employed to monitor the dough's temperature, which was maintained at 30°C under normal conditions. However, during CO_2 mixing tests, the temperature was lowered to 25°C to enhance the solubility of CO_2 in the dough, as it contains a higher proportion of water compared to fat.

The LB-ABC dough was prepared with the following procedure: 1 min of pre-mixing with a tool speed of 50 rpm and air flushing, followed by 1 min of mixing at atmospheric pressure at a tool speed of 100 rpm, and 8 min of kneading at atmospheric pressure at a tool speed of 100 rpm. The satellite speed was set to 5 rpm during pre-mixing and mixing, and 10 rpm during kneading. The temperature was maintained at 30°C throughout the process.

For the CO_2 mixing procedure, LB-CO2, dough was prepared using the following steps: 1 min of pre-mixing with partial vacuum at -0.9 bar with a tool speed of 50 rpm, followed by 2 min of mixing at 0.5 bar with a tool speed of 100 rpm, and 10 min of kneading at 0.5 bar with a tool speed of 180 rpm.

The satellite speed was set to 5 rpm during pre-mixing, 5 rpm during mixing, and 10 rpm during kneading.

After resting for 30 min at 27°C, the LB-ABC and LB-CO2 doughs were passed through a RONDO dough sheeter (RONDO, Germany). The lamination process occurred in five stages, with nip sizes of 10, 8, 6, 4, and 2 mm between the rolls. Following lamination, the dough was manually shaped using a 60 × 60 mm rectangular cutter equipped with needles. The biscuits were then arranged on an oven rack for baking, with 28 biscuits baked at a time.

- **Rotary Biscuit Dough**

The creaming method was used to prepare the rotary biscuit dough. A KENWOOD mixer (France) equipped with a flat K-shape beater was utilized for the mixing process. The initial mixing procedure, developed by the CTCPA Technical Centre (France), was later adapted for dough production using the forming machine. The process began by adding butter and sugar to the mixer, which were mixed for 10 min at 106 rpm. Afterwards, ABC, dissolved in water, was added to the mixture, followed by salt and milk powder. The mixing continued for an additional 2 minutes at 106 rpm. In the final phase, SBC, SAPP 10, and flour were incorporated and mixed for 2 more min at 106 rpm.

After mixing, the dough was allowed to rest for 15 min at ambient temperature before forming. The rotary biscuit dough was then shaped using a rotary moulder (PMP food machinery, Italy), provided by the CTCPA Technical Centre (France) at a speed of 4. The resulting biscuits were rectangular in shape, measuring 50 × 39 mm with a thickness of 2 mm. The biscuits were arranged on an oven rack for baking, with a total of 55 biscuits baked at a time.

4.4.7.2. Baking

- **Conventional Baking**

The LB-ABC-CB and LB-CO2-CB biscuits were baked in a deck oven (MIWE, Germany) at 240°C (top) and 250°C (bottom) for 9 min and 30 s. For the LB-SB-CO2 dough, the baking time was extended to 10 min to prevent the formation of cracks, which occur due to uneven water distribution in the dough during baking. After baking, 8 biscuits from each batch were selected and marked for further analysis. To simulate industrial baking conditions, 140 mL of saturated steam was injected into the oven during the first 2 min of baking. This steam helped delay crust formation, allowing time for water evaporation and improving the oven rise. The RB-ABC-CB biscuits were baked at 220°C for 6 min and 30 s in a MIWE deck oven (Germany). The internal temperature changes of laminated biscuits were monitored and registered using attached sensors and a data logger (OMEGA, Taiwan). After baking, the biscuits were

allowed to cool at ambient temperature for 30 min before being sealed in a plastic bag for further analysis.

- **Microwave Baking**

LB-ABC-MW and RB-ABC-MW were baked using a microwave oven. Microwave baking tests were carried out at the CTCPA Technical Centre in Avignon, France. Both laminated and rotary biscuits were made at GEPEA laboratory and were frozen at -18°C, 4 weeks before the baking tests. For the baking tests, the dough pieces were left on a baking tray for thawing for around 1 hr, even though the amount of frozen water was very low. Then dough pieces were installed on the baking support, which was made from BBQ baking net coated with PFAS coating (Teflon®, Dupont). The baking net was installed on the conveyor belt of the SAIREM prototype microwave oven (France), which was used for the industry baking tests. The microwave oven was equipped with 8 magnetrons, operating at 2450 MHz. Each magnetron had a nominal power of 2 kW (Figure 4.29).

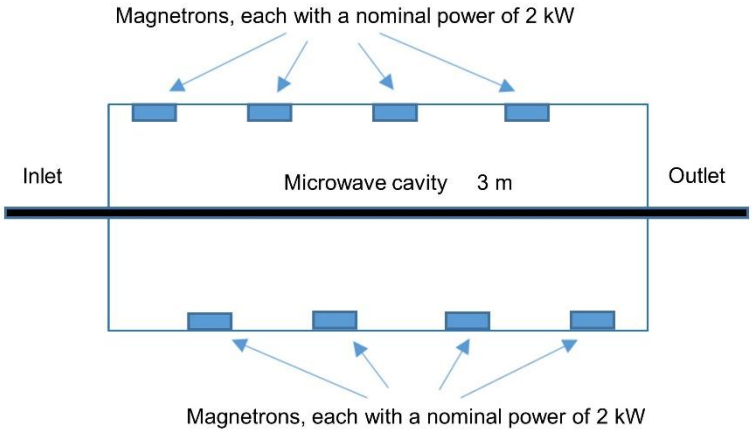

Figure 4.29. SAIREM prototype microwave oven schema used for the industry baking tests. It is equipped with 8 magnetrons with a nominal power of 2 kW each.

The power emitted by each magnetron (PXT and PXB) is summarised in Table 4.21 for both types of biscuits. A total power of 3.4 kW and 2.8 kW was applied to laminated and rotary biscuits, respectively. The conveyor speed and residence time were 37 cm/min and 8 min, respectively. During baking, changes in the temperature of the biscuits were monitored using optical fibres. Since microwave baking is not 100% efficient and only part of the power is absorbed by the food in the cavity, the reflected power (RP) was measured as well. In case of combined microwave-conventional baking (LB-ABC-MW-CB and RB-ABC-MW-CB biscuits), microwave baking was followed by 2 min of baking in a conventional oven (AKZ96490 IX, Whirlpool, France) at 250°C.

Table 4.21. Power values and specific energy for baking of laminated and rotary biscuits.

Type of dough	P1T	P1B	P2T	P2B	P3T	P3B	P4T	P4B	PT	RP1	RP2	PT-PR	SE
Laminated	0.3	0.3	0.3	0.3	0.5	0.5	0.6	0.6	3.4	0.14	0.29	2.97	1.41
Rotary	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	2.8	0.1	0.2	2.5	1.18

(SE: specific energy in MJ/kg dough; PXT: power of the top magnetron; PXB: power of the bottom magnetron; PT: total power; RP: reflected power).

4.4.8. References

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4.5. *In vitro* starch digestibility and estimated glycaemic index (eGI)

In this part, the results of *in vitro* starch digestibility and eGI for laminated and rotary biscuits baked using conventional, microwave, and combined conventional-microwave methods are presented. The effects of both the baking method and formulation are also discussed.

4.5.1. Resistant (RS) and digestible starch (DS)

The RS and DS contents of the biscuits were measured by an external laboratory, and the results are presented in the table below (Table 4.22):

Table 4.22. The resistant starch (RS), digestible starch (DS), and total starch (TS) contents of biscuits.

Type of biscuit	RS (% w/w)	DS (% w/w)	TS (% w/w)
LB-ABC-CB	0.3	57.2	57.5
LB-CO2-CB	0.3	57.9	58.2
LB-ABC-MW-CB	< 0.3	58.3	~58.6
RB-ABC-CB	< 0.3	47.8	~48.1
RB-ABC-MW-CB	< 0.3	49.1	~49.4

(LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10).

It can be observed that, despite the use of different formulations and baking methods, the RS content of biscuits remained relatively similar across the samples.

4.5.2. Degree of starch hydrolysis

Over the course of 20, 30, 60, 90, 120, 150, and 180 min, the degree of starch hydrolysis in the biscuit samples was measured (Figure 4.30). The results show irregular fluctuations in hydrolysis over time in all five samples, indicating that starch broke down inconsistently as the incubation period increased.

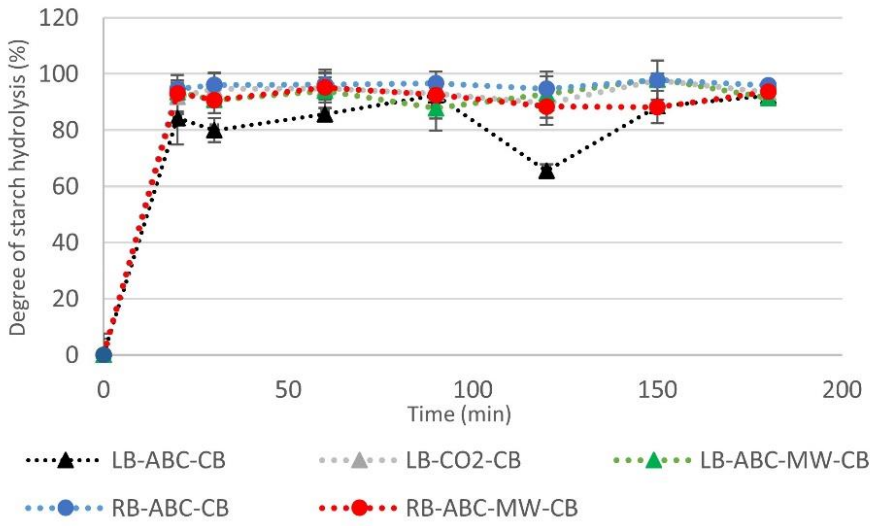

Figure 4.30. Partially hydrolysed starch over time; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10).

These sharp decreases observed in the degree of starch hydrolysis may be linked to limitations in the *in vitro* measurements using enzymatic kits. This can be justified by similar formulations and processes applied to products, so the differences between the products may be too small to be determined with enzymatic kits. In addition, when RS values are close, the sensitivity of *in vitro* methods may not be sufficient to capture slight differences in hydrolysis. Therefore, *in vivo* measurements could be a more accurate method to investigate the degree of starch hydrolysis in biscuits.

4.5.3. Estimated glycaemic index (eGI) of biscuits

The eGI of the biscuits was measured at 90 min. The results demonstrate that the GI ranged from 80.10 ± 3.63 in LB-ABC-MW-CB to 87.95 ± 7.22 in RB-ABC-MW-CB. ANOVA results revealed that, with a 95% confidence interval, none of the pairwise comparisons were significantly different.

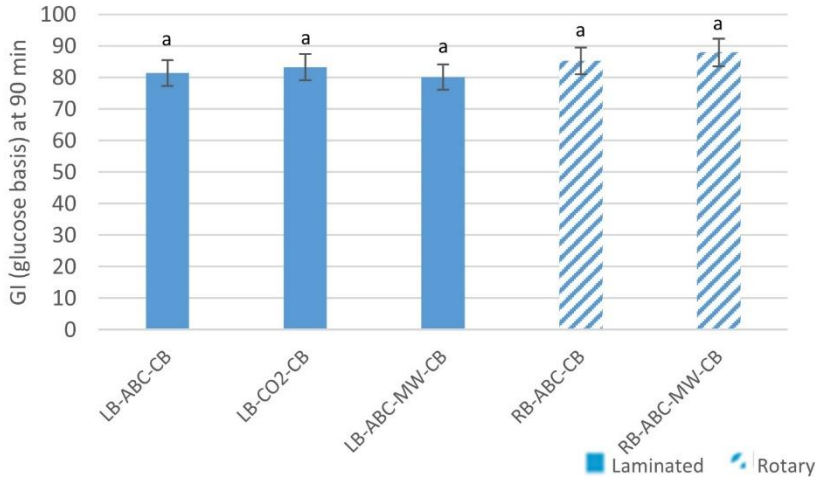

Figure 4.31. eGI of biscuits; (LB-ABC-CB: conventionally-baked laminated biscuit containing ABC, SBC, and SAPP 10 (as a control for laminated biscuits); LB-CO2-CB: conventionally-baked CO_2-mixed laminated biscuit free from ABC, containing SBC, and SAPP 10; LB-ABC-MW: microwave-baked laminated biscuit containing ABC, SBC, and SAPP 10; LB-ABC-MW-CB: combined microwave-conventionally baked laminated biscuit containing ABC, SBC, and SAPP 10; RB-ABC-CB: conventionally-baked rotary biscuit containing ABC, SBC, and SAPP 10 (as a control for rotary biscuits); RB-ABC-MW: microwave-baked rotary biscuit containing ABC, SBC, and SAPP 10; RB-ABC-MW-CB: combined microwave-conventionally baked rotary biscuit containing ABC, SBC, and SAPP 10); bars with the same letter are not significantly different (95% confidence interval).

According to the results, the formulation and baking method did not significantly affect the GI contents of the biscuits. This can be attributed to the similar RS content observed across all biscuits, as presented in the first section. Although the GI of the laminated biscuits baked using the combined microwave-conventional method (LB-ABC-MW-CB) was slightly lower than that of the conventionally-baked one (LB-ABC-CB), this difference was not statistically significant. The slight reduction may be linked to the influence of microwave baking on RS content, though the secondary baking method (conventional baking), may have diminished this effect. This can be explained by the effect of microwave baking on the RS; this difference was not significant, maybe due to applying the second baking (conventional). The suppression of ABC did not significantly affect the GI content. The GI contents of rotary biscuits (RB-ABC-CB and RB-ABC-MW-CB) were slightly higher compared to those of the laminated biscuits. This difference could be linked to the higher sugar content in the rotary biscuits—35.24 g—compared to the laminated biscuits, which contained 31.85 g of sugar and 2.23 g of glucose syrup or other parameters like dry matter, or even amylose content.

Sánchez-Pardo et al. (2007) compared the RS of microwave-baked pound cake crumbs with those baked conventionally. They observed the RS content of 4.4% in microwave-baked pound cake, which was significantly different ($P \leq 0.05$) from that in conventionally-baked pound cake (3.6%). They reported that differences in RS content are likely attributed to the fact that the microwave heating delivers rapid energy input to the product's centre, leading to considerable evaporative water loss that

does not occur with other cooking methods. Although the required temperature is achieved, gelatinisation does not occur completely in microwave-heated samples due to limited starch–water interaction due to the short microwave heating period. The higher RS content in microwave-baked pound cake may indicate the presence of either higher levels of non-gelatinised starch or retrograded starch, as RS includes both of them.

These findings contrast with the results observed in our study. This could be due to different factors. Firstly, the product matrix used in their study- a pound cake- was different from that used in ours (biscuits). Secondly, the microwave baking of pound cakes was done at a power of 240 W for 5 min, whereas the biscuits were baked at 2.8 kW-rotary biscuits and 3.4 kW-laminated biscuits. Additionally, they did not combine the microwave and conventional baking. The second baking step (conventional baking) in our process may have further influenced the RS content of the final products. Furthermore, apart from the baking method, the starch digestibility and GI are impacted by a variety of intrinsic and extrinsic factors. Proteins, lipids, fibre, and high amylose content have all been demonstrated to slow down the rate of starch hydrolysis (Lal et al., 2021).

Foods are categorised as low GI (GI ≤ 55), medium GI (56 ≤ GI ≤69), or high GI (GI ≥ 70) (Priyadarshini et al., 2022; Venn & Green, 2007). Therefore, the tested biscuits are classified in high GI category.

By increasing the internal temperature of cookies, longer baking times can slow down the rate of starch hydrolysis and improve starch retrogradation when the cookies cool. Improved starch gelatinisation is another benefit of this process. Higher baking temperatures (140–160°C) promote the development of complexes between lipids and amylose that are difficult to digest. Extended baking also changes the structure of the dough by acting as a heat-moisture treatment. Starch's digestibility is eventually impacted by this process as the breakdown of native molecular bonds and the creation of more resistant structures cause it to lose some of its birefringence (Naseer et al., 2021).

4.5.4. Conclusion

The findings presented above indicate that combined microwave-conventional baking did not significantly affect the RS of the biscuits. Although previous studies, like the one conducted by Sánchez-Pardo et al. (2007), have shown that microwave baking can reduce the GI by increasing the RS content, this effect was not observed in the current study. One possible explanation is the influence of the subsequent conventional baking step, which was applied to improve the texture and colour of the biscuits and may have diminished the impact of microwave baking on RS formation. Therefore, it would be interesting to assess the GI of biscuits baked using microwave alone, for both laminated and rotary

types. Additionally, due to the limitations of the *in vitro* method used in this study, *in vivo* measurements are recommended for a more accurate evaluation of the biscuits' GI.

4.6. Fibre-added laminated biscuits

The impact of adding acorn and raw carob flours to wheat-based biscuit formulations is examined in this part of the Results and Discussion, with particular attention paid to how these additions affect the physicochemical, functional, and sensory qualities of the flours and the finished biscuits. These flours were selected based on their unique lipid, fibre, and starch profiles. This part aims to evaluate the suitability of these substitute flours for biscuit production and their potential to increase nutritional value by evaluating functional performance and sensory results. The primary goal is to assess how important quality indicators like water content, bulk density, water and oil holding capacities, swelling capacity, texture, colour, and consumer acceptability are impacted by partial substitution of wheat flour (at 20% and 30% levels). The tested biscuits are: the control biscuit (LB-ABC-CB with 100% wheat flour), LB-AC-20 (20% of wheat flour replaced by acorn flour), LB-AC-30 (30% of wheat flour replaced by acorn flour), LB-CA-20 (20% of wheat flour replaced by raw carob flour), and LB-CA-30 (30% of wheat flour replaced by raw carob flour). All biscuits were baked conventionally.

4.6.1. Physical and functional properties of acorn and raw carob flours

The physical and functional properties, including water content, bulk density, water holding capacity (WHC), oil holding capacity (OHC), and swelling capacity (SWC), of the control (wheat flour), acorn flour, and raw carob flour, measured in triplicate, are shown below (Table 4.23):

Table 4.23. Physical and functional properties of acorn and raw carob flours; (AC: acorn flour; CA: raw carob flour; WHC: water holding capacity; OHC: oil holding capacity; SWC: swelling capacity; values with the same letter in the same column are not significantly different (95% confidence interval).

Type of flour	Water content (% d.b)	Bulk density (g/mL)	WHC (g water/g dry sample)	OHC (g water/g dry sample)	SWC (mL/g dry sample)
Control (wheat flour)	15.65 ± 0.05 ^{a}	0.46 ± 0.01 ^{a}	0.67 ± 0.04 ^{a}	0.93 ± 0.04 ^{a}	14.06 ± 0.31 ^{a}
AC	14.29 ± 0.15 ^{b}	0.45 ± 0.00 ^{b}	1.78 ± 0.16 ^{b}	1.00 ± 0.03 ^{a}	3.97 ± 0.50 ^{b}
CA	10.28 ± 0.08 ^{c}	0.76 ± 0.01 ^{c}	1.43 ± 0.02 ^{c}	1.10 ± 0.15 ^{a}	3.36 ± 0.17 ^{b}

The highest value of water content was observed for wheat flour (15.65%, d.b.) and the lowest for raw carob flour (10.28%, d.b.). Flours with low water content (< 10%) are preferred, as lower water content

extends shelf-life and reduces bulk, therefore making the material easier to package, handle, and transport (Rana et al., 2015).

ANOVA revealed a significant difference among the bulk densities. Acorn flour had the lowest bulk density (0.45 g/mL), and raw carob flour had the highest bulk density (0.76 g/mL). In studied flours, bulk density is mainly influenced by particle size and packing characteristics. Lipids can increase packing efficiency, as triglycerides may act as binders, increasing agglomeration of protein and carbohydrate molecules, which in turn raises bulk density (Dogruer et al., 2023). Differences in bulk density may be related to variations in their starch content, with higher starch levels often leading to greater bulk density. Water content at the time of processing can also influence how densely a flour packs. In general, flours with higher bulk density are considered more suitable for food applications, as they are easier to store and handle (Godswill et al., 2019).

WHC ranged from 0.67 to 1.78 g water/g dry sample, with wheat flour showing the lowest value and acorn flour the highest. Starch accounts for the largest share, binding roughly 46% of the total absorbed water. Proteins contribute about 31%, while pentosans are responsible for around 23%. The addition of vital wheat gluten further enhances water absorption and contributes to improved dough stability. Other water-binding components, such as fibre, bran, hydrocolloids (like gums), and eggs, can also increase the flour's ability to absorb and retain water (Godswill et al., 2019). WHC indicates how much water is available for starch gelatinisation (Elkhalifa et al., 2005).

The OHC of flours ranged from 0.93 to 1.10 g oil/g dry sample across all types of individual flours. It was highest in raw carob flour (1.10 g oil/g dry sample) and lowest in wheat flour (0.93 g oil/g dry sample). However, ANOVA did not reveal a significant difference among the samples. OHC is a crucial functional property that enhances mouthfeel and preserves food flavour. Protein-rich foods tend to absorb oil more readily, influenced by intrinsic protein characteristics such as conformation, amino acid structure, and surface polarity or hydrophobicity. Flours with strong oil-binding properties are especially valuable in food formulations requiring optimal oil absorption. The protein structure, with its hydrophobic and hydrophilic poles, primarily governs oil absorption capacity. Non-polar amino acid side chains can interact hydrophobically with lipid hydrocarbon chains. Capillary action within the food matrix also plays a significant role in retaining absorbed oil (Godswill et al., 2019).

The value of SWC was found to be highest for wheat flour (14.06 mL/g dry sample) followed by acorn flour (3.97 mL/g dry sample) and raw carob flour (3.36 mL/g dry sample). Statistically, there was no significant difference between the SWC of acorn and raw carob flours. The term swelling capacity describes the starch's capacity to absorb water in flour and expand, which is crucial for assessing the quality of products like baked goods (Chiranthika et al., 2024). It is influenced by the ratio of amylose

to amylopectin, with higher amylopectin content generally increasing swelling. Particle size, plant variety, and processing techniques are some of the other variables that impact this property. Since the composition of starch granules varies depending on the plant source, different flours have varying capacities for swelling. This functional characteristic is also significantly influenced by non-covalent interactions that occur within starch molecules (Godswill et al., 2019). As starch gelatinization is inhibited, flours with higher protein and fat contents typically have less swelling power. It is observed that the bread made from composite flour variants had a lower swelling index, which could have been caused by the observed decrease in loaf volume and height (Lakshmi et al., 2015). Dietary fibre can prevent the gelatinisation process, which in turn can prevent the swelling of starch granules. This may result in a decrease in the flour's overall capacity to swell (Chiranthika et al., 2024).

The colour of flours was measured and depicted in the table below (Table 4.24):

Table 4.24. Flours' colour values and colorimetric distances between the control and acorn and raw carob flours.

Type of flour	L*	a*	b*	ΔE
Control (wheat flour)	95.83 ± 0.07 [a]	0.15 ± 0.02 [a]	9.31 ± 0.26 [a]	0.00
AC	57.45 ± 0.56 [b]	7.65 ± 0.06 [b]	26.41 ± 0.08 [b]	42.67
CA	48.09 ± 0.36 [c]	12.28 ± 0.08 [c]	26.49 ± 0.04 [b]	52.16

(AC: acorn flour; CA: raw carob flour; values with the same letter in the same column are not significantly different (95% confidence interval).

The results show that acorn and raw carob are significantly darker than wheat flour (Figure 4.32). ΔE value is commonly used to compare samples and determine whether any colour difference would be noticeable to the human eye. Since ΔE in acorn and raw carob flours was greater than 12, the colour difference is noticeable. These two flours are significantly darker than wheat flour. In addition, their a* and b* values are higher, suggesting a more pronounced reddish and yellowish hue compared to wheat flour.



Figure 4.32. Acorn and raw carob flours.

4.6.2. Dough texture

Firmness and deformation of doughs made by replacing different percentages of wheat flour with acorn and raw carob flours were measured using TPA (Figure 4.33). The incorporation of both acorn and raw carob flours significantly increased the firmness of doughs compared to the control. This is in accordance with the findings of Martins et al. (2020). They observed that acorn flour in gluten-free bread recipes had a major impact on the rheological characteristics and dough quality. Acorn flour increased the dough's cohesiveness, elasticity, and firmness at moderate substitution levels (23%), suggesting better structural integrity. These results are mostly attributable to the high dietary fibre content of acorn flour, especially the insoluble fibre, which increases firmness by promoting better water absorption and a denser dough structure. The firmness of structure was somewhat reduced at higher substitution levels (35%), most likely as a result of the starch being diluted and the dough matrix being disrupted. Acorn flour also increased the amount of total and insoluble dietary fibre in the dough. The higher firmness of LB-CA-20 and LB-CA-30 could be linked to the higher fibre content of this flour compared to acorn flour.

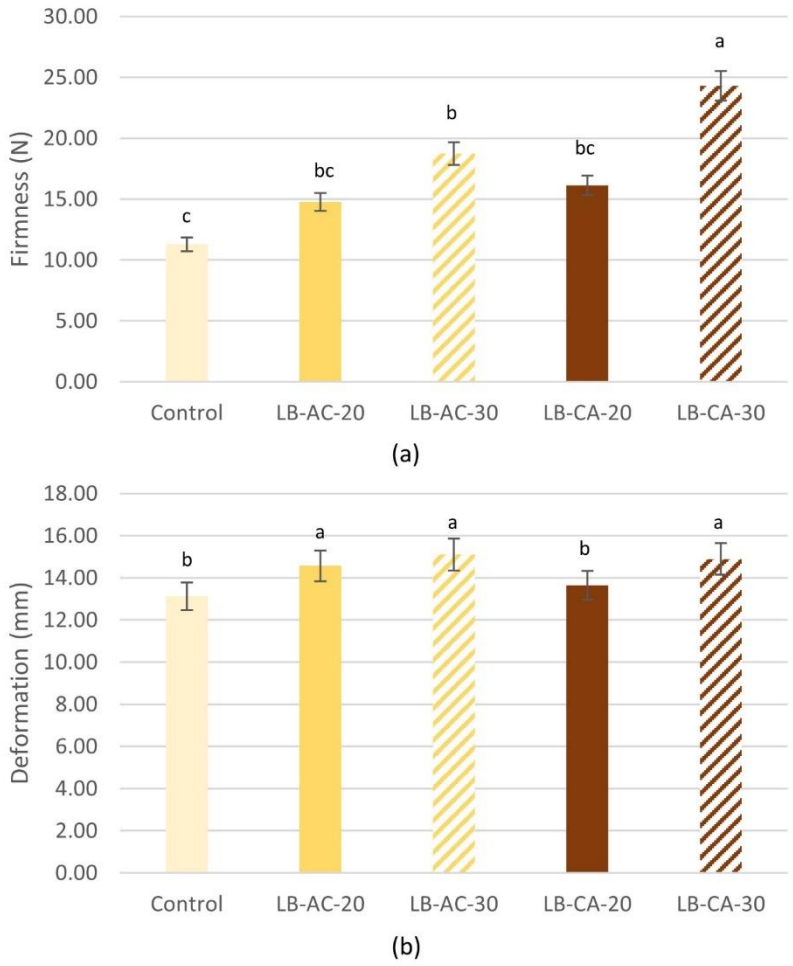

Figure 4.33. (a) Firmness and (b) deformation of doughs containing 20% and 30% of acorn and raw carob flours; (LB-AC-20: laminated biscuit dough with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit dough with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit dough with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit dough with 30% of wheat flour replaced with raw carob flour; bars with the same letter are not significantly different (95% confidence interval).

4.6.3. Fibre-added biscuits characteristics

Physical characteristics of the fibre-added biscuits are presented in the table below (Table 4.25).

Table 4.25. Physical characteristics of fibre-added biscuits.

Type of biscuit	Water content % (d.b)	Specific volume (cm^3/g)	Hardness (N)	L*	a*	b*	ΔE
Control	3.16 ± 0.17 ^{a}	3.47 ± 0.02 ^{a}	27.56 ± 3.57 ^{a}	62.51 ± 0.59 ^{a}	10.22 ± 0.02 ^{a}	28.79 ± 0.39 ^{a}	0.00
LB-AC-20	2.64 ± 0.06 ^{b}	3.15 ± 0.05 ^{b}	20.71 ± 2.50 ^{b}	56.43 ± 0.56 ^{b}	7.20 ± 0.30 ^{b}	26.19 ± 0.46 ^{b}	7.29
LB-AC-30	2.36 ± 0.22 ^{b}	2.84 ± 0.03 ^{c}	15.60 ± 3.12 ^{c}	54.16 ± 0.48 ^{c}	6.61 ± 0.25 ^{c}	24.62 ± 0.66 ^{c}	10.03
LB-CA-20	3.40 ± 0.44 ^{a}	2.70 ± 0.14 ^{d}	18.91 ± 2.86 ^{b}	52.20 ± 0.63 ^{d}	11.19 ± 0.15 ^{d}	18.33 ± 0.67 ^{d}	14.72
LB-CA-30	3.56 ± 0.16 ^{a}	2.60 ± 0.04 ^{e}	23.30 ± 2.96 ^{ab}	47.77 ± 0.57 ^{e}	10.87 ± 0.06 ^{d}	15.09 ± 0.52 ^{e}	20.13

(LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour; values with the same letter in the same column are not significantly different (95% confidence interval).

The biscuits had a water content ranging from 2.36% to 3.56% (d.b.). Even though the biscuits made with raw carob flour (LB-CA-20 and LB-CA-30) took less time to bake than the control (8 min and 30 s vs. 9 min and 30 s), there was no significant difference between the two. This can be explained by the raw carob biscuits' high fibre content. LB-AC-20 and LB-AC-30 biscuits made with acorn flour had a substantially lower water content than the raw carob and control biscuits.

The samples' specific volumes varied from 2.60 to 3.47 cm^3/g over the samples. ANOVA showed that the samples differed significantly. Acorn and raw carob flours were found to significantly reduce the biscuits' specific volume when their percentage was raised from 20% to 30%. These results are in accordance with the earlier findings. In a study conducted by Papageorgiou et al. (2020), wheat flour of a cake was substituted by roasted carob flour at 10%, 30%, 50%, and 70% and they were compared with the control cake. They observed that the specific volume of the cakes decreased as the carob flour substitution increased. This is attributed to the presence of fibre in biscuits, because Increased fibre amounts deteriorate the capabilities of dough due to the dilution of the gluten network, which in turn reduces the gas retention capacity of dough (Papageorgiou et al., 2020; Tsatsaragkou et al., 2014). Similar findings were reported by Molavi et al. (2015) on the incorporation of acorn flour into the sponge cake.

The control has the highest hardness value (27.56 N), and there was no significant difference between that and the biscuit containing 30% raw carob flour (LB-CA-30). It was observed that by increasing the acorn flour from 20% to 30%, the hardness decreased from 20.71 N to 15.60 N. The same observations was reported by Pasqualone et al. (2019) when they incrorporated 30% and 60% acorn to biscuits. The decrease in biscuit hardness with increasing levels of acorn flour is mainly attributed to the dilution and weakening of the gluten network. Since acorn flour is naturally gluten-free, replacing part of the wheat flour reduces the amount of gluten-forming proteins available in the dough. As a result, the biscuit matrix develops less structural cohesion, leading to a more brittle and friable texture after baking. In addition, acorn flour contains a relatively high amount of fats, which further interfere with gluten development by coating the protein molecules (Pasqualone et al., 2019). However, our findings were in contrast with the findings of Szabłowska and Tanska (2016). They evaluate the impact of the incorporation of acorn flour from 5% to 50% in bread. They observed that by increasing the acorn the crumb hardness increased. Regarding the carob flour, a study conducted by Papageorgiou et al. (2020) observed that the addition of roasted carob flour affected the texture of the cakes, depending on the amount used. When 10% or 30% of the wheat flour was replaced with roasted carob flour, the cakes became softer than the control cocoa cake. However, when the replacement level reached 50%, the cakes became significantly harder. This difference can be justified by the different matrix that we used, as we studied biscuits, which have less water compared to cakes.

Visual differences in biscuit colour were significant. The incorporation of higher proportions of acorn and carob flours resulted in darker biscuits. This is linked to the dark colour of acorn and raw carob flours in addition to the Maillard reaction, and it aligns with previous findings (Pasqualone et al., 2019; Szabłowska & Tanska, 2016). According to ΔE values, the colour difference of fibre-added biscuits would be noticeable to the human eye, as they are greater than 6 (Lo Faro et al., 2022). From a sensory standpoint, such obvious variations in appearance could affect consumer appreciation because darker colour hues are frequently linked to higher fibre content, roasted flavour notes, or artisanal quality.

Fibre and protein contents of fibre-added biscuits were measured by an external laboratory and reported (Table 4.26).

Table 4.26. Nutritional composition of fibre-added biscuits.

Type of biscuit	Protein (g / 100 g biscuit)	TDF (g / 100 g biscuit)
Control	6.3 ± 0.5	2.3 ± 1.1
LB-AC-20	6.0 ± 0.5	4.1 ± 1.4
LB-AC-30	6.0 ± 0.5	4.5 ± 1.5
LB-CA-20	6.3 ± 0.5	7.4 ± 1.8
LB-CA-30	5.7 ± 0.5	9.5 ± 2.0

(TDF: total dietary fibre; LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour).

According to the findings, the protein content of all biscuit samples was almost similar. However, the differences in total dietary fibre became obvious with the addition of acorn and raw carob flours. The fibre content of the laminated biscuit increased by 1.78 times when 20% of the wheat flour was replaced with acorn flour (LB-AC-20), while this value was 3.22 for 20% raw carob flour substitution (LB-CA-20). When 30% of the wheat flour was replaced, the fibre content increased by 1.96 times for acorn flour (LB-AC-30) and 4.13 times for raw carob flour (LB-CA-30).

According to Regulation (EC) No 1924/2006, a product is considered a “source of fibre” if it contains at least 3 g fibre/100 g, and “high in fibre” if it contains at least 6 g fibre/100 g. Following these definitions, acorn biscuits are qualified as a “source of fibre” and carob biscuits (LB-CA-20 and LB-CA-30) meet the criteria to be labeled “high in fibre” or “rich in fibre”.

4.6.4. Sensory analysis

Five biscuits were included in the sensory analysis (Figure 4.34).

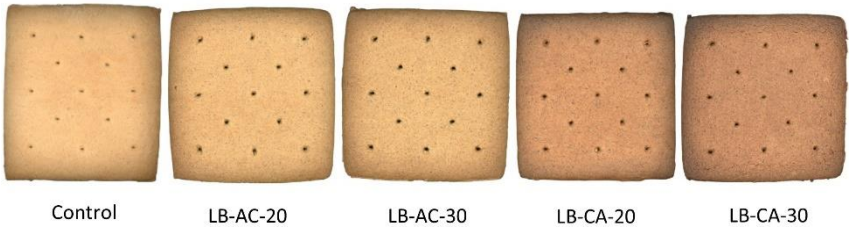

Figure 4.34. The control and fibre-added biscuits included in the sensory analysis; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour).

4.6.4.1. Hedonic liking score

The panellists' scores for overall liking showed statistically significant ($p < 0.05$) differences among biscuits (Figure 4.35). The highest score was for the control biscuit (6.61 ± 2.01), followed by those of LB-AC-20 and LB-AC-30 biscuits (6.08 ± 1.87 and 5.48 ± 1.94, respectively), being the lowest scores were those of LB-CA-20 and LB-CA-30 biscuits (4.44 ± 2.31 and 4.34 ± 2.70, respectively). Therefore, the incorporation of raw carob flour reduced the overall liking significantly ($p < 0.05$). The lower overall-liking scores of these biscuits could be related to changes in the taste, colour, hardness, and aroma intensity. Papageorgiou et al. (2020) showed that substituting wheat flour and cocoa with carob flour influenced the cakes' colour, sweetness, and texture, while most other attributes remained comparable to the control. As the level of carob flour increased, the cakes became progressively darker, with 30% and 50% replacement producing a colour similar to the cocoa cake, while 70% made the cakes noticeably darker. Sweetness slightly increased with higher carob content due to the natural sugars in carob, and cakes with 70% substitution were perceived as significantly sweeter than the control. In terms of texture, cakes containing up to 30% carob flour were rated as equally soft as the control, but those with 50% or more became firmer and less tender. Overall, cakes with 30% carob flour achieved the most favourable balance of colour, sweetness, softness, and aroma, resulting in the highest overall acceptability among the reformulated samples.

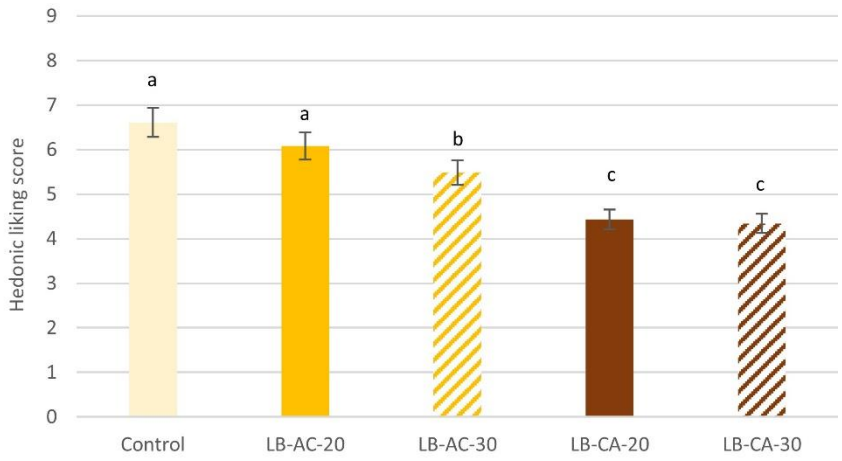

Figure 4.35. Hedonic liking score of fibre-added biscuits; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour; bars with the same letter are not significantly different (95% confidence interval).

4.6.4.2. Just-About-Right (JAR) scales

The results of JAR scales are demonstrated in Figure 4.36. The control sample showed mainly JAR score for all attributes except "cocoa aroma". This is due to the absence of cocoa or any other aroma similar

to cocoa in the control, which aligns with the formulation applied. However, the “cocoa aroma” attribute was still included for consistency in the questionnaire. 49% of panellists thought that the “aromatic intensity” was too little. 36% of the panellists thought that the hardness, and 34% thought the crumbliness of the control biscuit were “too much”.

At 20% acorn flour substitution (LB-AC-20), the biscuits maintained good overall sensory balance, similar to the control, but with slightly softer and less crumbly textures. Around 60% of participants found the hardness and crumbliness JAR score, indicating an acceptable bite and structure. Sweetness remained well balanced (61% JAR). Cocoa aroma improved compared with the control; however, most people found it “too little”. In a study conducted by Pasqualone et al. (2019), it was found that biscuits with acorn flour were perceived to have odor sensations such as “bran-like” and “caramel notes” rather than cocoa. The perception of cocoa aroma could be explained by the darker colour of biscuits containing acorn flour. Overall, LB-AC-20 biscuits were perceived as well-balanced, maintaining good texture and flavour harmony.

Increasing acorn flour to 30% (LB-AC-30) slightly altered the sensory profile. Graininess (84%) and bitterness (79%) received the largest percentage of JAR responses, indicating that these qualities are highly optimised and closely match panellists’ expectations. However, most people thought that characteristics like colour (55% rated it as “too dark”), consistent with acorn’s deep pigment, and aromatic intensity (54% rated it as “too little”) were “too much” and “too little” , respectively, suggesting that an improvement in the formulation is needed. Sweetness was balanced, as 49% of panellists rated it as being JAR. Hardness (68% JAR) was generally acceptable but crumbliness showed 52% “too high” responses suggesting slightly excessive textural instability. In order to create a more balanced sensory profile LB-AC-30, optimisation efforts should generally concentrate on improving colour and aromatic intensity.

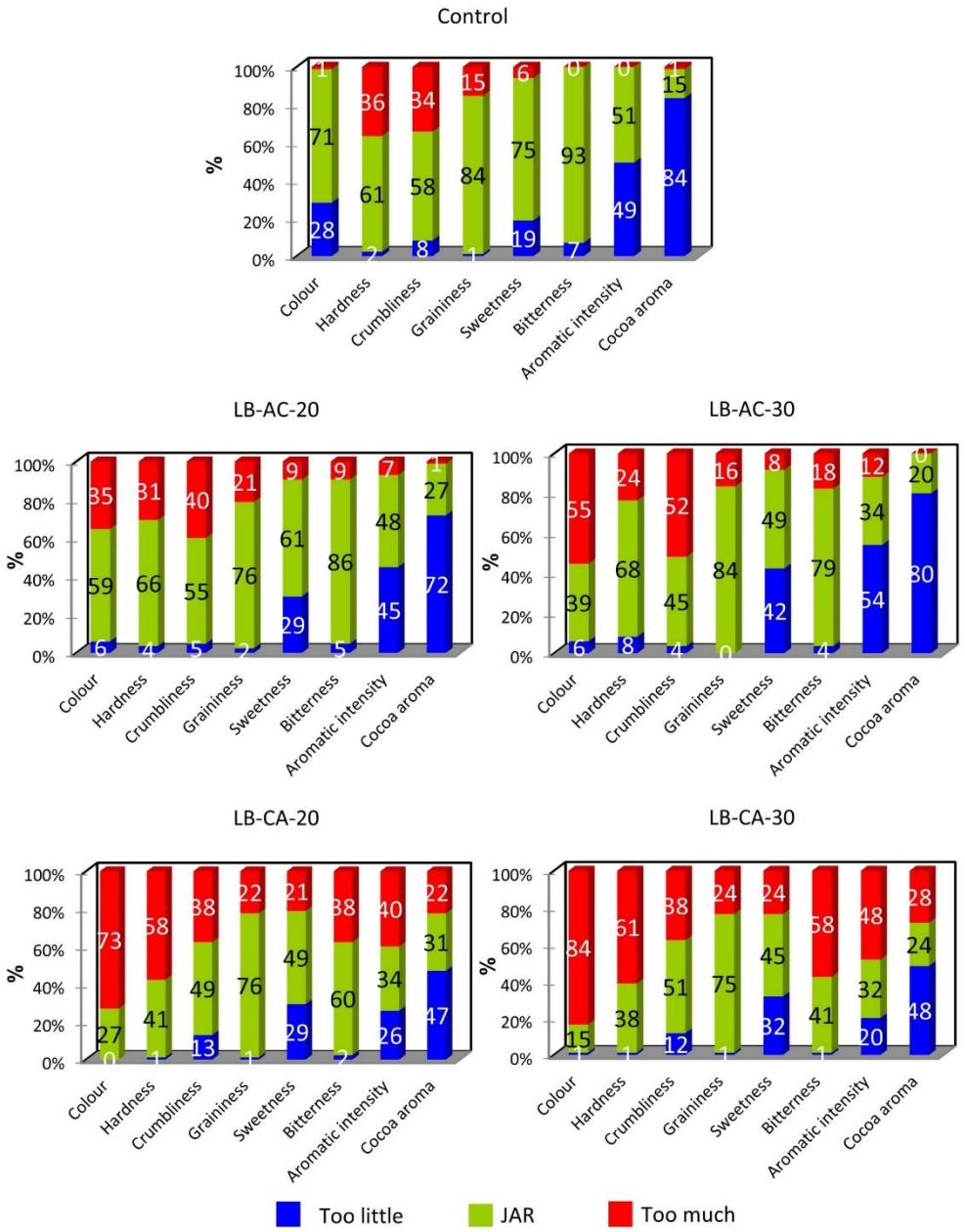

Figure 4.36. Just-About-Right (JAR) scale percentages of responses grouped in three levels; (LB-AC-20: laminated biscuit with 20% of wheat flour replaced with acorn flour; LB-AC-30: laminated biscuit with 30% of wheat flour replaced with acorn flour; LB-CA-20: laminated biscuit with 20% of wheat flour replaced with raw carob flour; LB-CA-30: laminated biscuit with 30% of wheat flour replaced with raw carob flour).

The biscuits containing 20% raw carob flour (LB-CA-20) differed substantially in sensory perception. Colour was perceived as "too dark" by about 70% of panellists, consistent with carob's intense pigmentation. The hardness was rated "too much" by 58% of panellists, indicating a firmer bite than desired. Sweetness and bitterness were almost balanced— 49% JAR for sweetness and 60% JAR for bitterness. Overall, LB-CA-20 biscuits were darker and firmer than preferred, suggesting carob substitution at this level begins to negatively impact sensory balance.

At 30% raw carob flour (LB-CA-30), deviations from the ideal sensory profile became more pronounced. The majority of panellists (over 80%) found the colour “too dark,” confirming a strong visual effect of raw carob. Hardness was again mostly rated “too much,” implying a dense texture. Sweetness was judged JAR by 45% of panellists, while bitterness and aromatic intensity were considered “too much” by many, likely due to carob’s natural bitter compounds, leading to a strong, slightly overbearing flavour profile. Cocoa aroma was also “too little” for 48% of panellists. Altogether, LB-CA-30 biscuits were viewed as overly dark, firm, and bitter, indicating that high carob flour levels negatively affect both texture and flavour acceptability.

Papageorgiou et al. (2020) observed that as the roasted carob increased, there was a noticeable rise in the perceived sweetness of the cakes. This increase was anticipated due to the sugar content of carob flour, which averages around 40% according to previous studies. Cakes with up to 30% carob flour substitution were considered to have a similar softness to the control. However, as the substitution level exceeded 30%, panellists perceived the cakes as becoming progressively less soft. In the studied biscuits, the increase of carob flour from 20% to 30% led to a slight increase in sweetness perception. This could be explained by the application of roasted carob flour in the study by Papageorgiou et al. (2020), which exerts a chocolate/cocoa-reminiscent flavour and colour. The increase in hardness of the biscuits was noted by the panelists with the increase of carob flour, even from 20% to 30%. This could be attributed to the difference in the food matrix, as biscuits contain less water compared to cakes.

4.6.5. Conclusion

Incorporation of acorn and raw carob flours substantially altered the rheological and textural behaviour of biscuit doughs. Dough firmness increased with the addition of both flours, consistent with their elevated fibre levels and limited gluten content. However, excessive substitution (30%) disrupted the gluten network, reducing cohesiveness and gas retention, thereby lowering the specific volume of the biscuits. Both acorn and raw carob flours imparted darker colours to the biscuits, attributable to their intrinsic pigments and the Maillard reaction during baking. These colour changes were detectable to the human eye, as indicated by high ΔE values.

From a compositional perspective, acorn and raw carob flours considerably enhanced the dietary fibre content of the biscuits. Acorn-based biscuits qualified as a “source of fibre”, whereas carob-based biscuits met the criteria to be labeled “high in fibre”, according to Regulation (EC) No. 1924/2006. Despite these nutritional advantages, sensory evaluation revealed that higher substitution levels adversely affected consumer acceptability. Biscuits containing 20% acorn flour maintained a sensory profile comparable to the control, whereas those with 30% acorn flour showed slight deviations in

colour and aromatic balance. Conversely, biscuits formulated with 20–30% raw carob flour were perceived as excessively dark, firm, and bitter, leading to significantly lower overall-liking scores.

Overall, the findings suggest that partial replacement of wheat flour with acorn or raw carob flour can enhance the nutritional quality of biscuits without severely compromising their technological or sensory attributes, provided the substitution level does not exceed 20%. Among the formulations tested, biscuits with 20% acorn flour achieved the most favourable balance between nutrition, texture, and sensory acceptance, making them the most promising alternative for developing fibre-enriched bakery products.

4.7. Energy consumption of baking

The baking energy of the biscuits was calculated according to the mathematical model discussed in Part 7 of the Materials and Methods. The calculations were performed using the formulations proposed in Section 1.2 of the Materials and Methods for both laminated and rotary biscuits. The results showed a total energy consumption of 901.60 J/g dough for the laminated dough and 690.91 J/g dough for the rotary dough. The lower energy consumption for baking rotary biscuits can be attributed to the differences in their formulations.

For the microwave baking of biscuits, following the assumptions described in Section 3.5.2 of the Results and Discussion, and assuming a microwave energy efficiency of 18.60% and 17.52% for laminated and rotary biscuits, respectively, the consumed energy was 516.68 J/g dough for laminated dough and 615.40 J/g dough for rotary dough.

Although microwave baking showed lower energy consumption for both laminated and rotary biscuits, it should be noted that the energy consumption computations for conventional baking were estimated using a theoretical mathematical model, while those for microwave baking were obtained experimentally. Therefore, it would be more appropriate to perform both calculations under comparable conditions to enable a more accurate comparison of their energy consumption.

4.8. Supplementary information

One artefact introduced by the presence of the LA is the mass loss (in the form of NH_3 gas) resulting from its decomposition. SBC is known to react within the temperature range of 35°C to 65°C, while ABC begins to degrade around 65°C. The corresponding reactions are as follows:

For ABC:

$$(NH_4)\,HCO_3 \rightarrow NH_3 + CO_2 + H_2O \quad \text{Eq. 4.9}$$

Ammonium Bicarbonate → Ammonia + Carbon dioxide + Water

For SBC:

$$NaHCO_3 + HX \rightarrow NaX + H_2CO_3 \quad \text{Eq. 4.10}$$

Sodium Bicarbonate + Acid → Neutral salt + Carbonic acid

$$H_2CO_3 \rightarrow H_2O + CO_2 \quad \text{Eq. 4.11}$$

Carbonic acid → Water + Carbon dioxide

The substances produced from these reactions appeared in either liquid or gaseous form within the dough. However, all of them eventually escaped as gases due to the final temperature of 140°C. It is expected that only a minor and negligible amount remained trapped in the baked biscuit. The molar masses of the different species involved in the LAs are provided in the table below (Table 4.27).

Table 4.27. Molar masses of the different substances involved in LAs.

Name	Formula	Molar mass (g/mol)
Sodium bicarbonate	$NaHCO_3$	84.01
Carbonic acid	H_2CO_3	62.03
Ammonium bicarbonate	$(NH_4)HCO_3$	79.06
SAPP 10	$Na_2H_2P_2O_7$	221.94
Ammonia	NH_3	17.03
Water	H_2O	18.01
Carbon dioxide	CO_2	44.01

In the case of laminated biscuits containg LAs, for 163.36 g of biscuit dough (which contains 100 g of flour in w.b.), the number of moles of LAs in the recipe is shown in Table 4.28. The SAPP 10 had no

significant influence; it primarily served to neuralise the SBC. The neutralization reaction leaves behind salts containing sodium and phosphorus in the baked biscuit.

Table 4.28. Moles of LAs in 163.36 g of laminated biscuit dough.

	Mass in the recipe (g)	Mole in the recipe (mol/g)	Mole/g dough
	163.36 (g dough based on 100 g flour (w.b.)		
ABC	1.00	1.26E-02	7.74E-05
SAPP 10	0.40	1.80E-03	1.10E-05
SBC	0.64	7.62E-03	4.66E-05

Based on the decomposition reaction of ABC, one mole of ABC yields one mole of NH_3, one mole of H_2O, and one mole of CO_2. This made it possible to determine the mass of the corresponding substances that would escape from the dough during baking, as well as their corresponding volumes, which were calculated at atmospheric pressure and an average temperature of 80°C using the ideal gas law (Table 4.29).

Table 4.29. Computed mass of the substances released from LAs during decomposition, along with the corresponding volumes per gram of dough (w.b.), using the ideal gas law at atmospheric pressure and a temperature of 80°C.

ABC	**Mole/g dough (w.b.)**	**Mass of substances g/g dough (w.b.)**	**Volume of substances Patm and 80°C (cm^3)**	
$C0_2$	7.74E-05	3.41E-03	2.24	
H_20	7.74E-05	1.39E-03	2.24	
NH_3	7.74E-05	1.32E-03	2.24	
Total		6.12E-03	6.73	
SBC	**Mole/g dough (w.b.)**	**Mass of substances g/g dough (w.b.)**	**Volume of substances Patm and 80°C (cm^3)**	
$C0_2$	4.66E-05	2.05E-03	1.34	
H_20	4.66E-05	8.39E-04	1.34	
Total		2.89E-03	2.68	
	Total ABC & SBC	9.01E-03	9.41	**% in mass**
	Total $C0_2$ ABC & SBC	5.46E-03	2.91	60.6%
	Total H_20 ABC & SBC	2.23E-03	2.91	24.7%
	Total NH_3 ABC	1.32E-03	2.24	14.7%

The results show that a total of 9.01 mg of CO_2, H_2O, and NH_3 were released per gram of dough (initial mass before baking in w.b.), with 6.12 mg originating from ABC and 2.89 mg from SBC. The corresponding total gas volume was 9.41 cm^3 per gram of dough (6.73 cm^3 and 2.68 cm^3 for ABC and SBC, respectively). Considering that 1 g of degassed dough corresponds to a volume of approximately

0.8 cm^3 (ρ = 1.37 g/cm^3), it can be observed that the volume of gas released by the LAs is about ten times greater than the dough volume. This suggests that a significant amount of the released gas from the LAs, escapes during baking.

5. CONCLUSION

5.1. General conclusion

This PhD project investigated the formulation, mixing, baking, and nutritional aspects of laminated and rotary biscuits, with a focus on reducing or eliminating chemical leaveners, exploring CO_2 over-pressure mixing, applying microwave baking, and enhancing nutritional quality by reducing the GI of and increasing dietary fibre of the biscuits. Therefore, the PhD objectives were:

1) Adapt the formulation and process through mixing under CO_2 to reduce or eliminate LAs
2) Develop an alternative baking strategy based on microwaves
3) Considering the impact of CO_2 over-pressure mixing and microwave baking of biscuits on the AA level
4) Evaluate the impact of the baking process on the eGI of the biscuits
5) Develop nutritionally improved biscuit formulations, notably by introducing acorn and carob flours to the biscuits to enhance the fibre content, and evaluate the impact of reformulations and processes on consumer acceptance.
6) Assessing the energy consumption of baking for both microwave and conventional baking

The main findings are as follows:

Enilia (T55) flour was identified as an appropriate flour for achieving biscuits with industrially acceptable density. The kneading conditions and baking parameters were optimised to balance dough development, gas retention, and surface quality. Incorporating CO_2 during mixing improved biscuit expansion and texture compared to no leaveners, but did not fully match the performance of chemical leaveners, indicating that further optimisation of CO_2 mixing is needed.

Microwave baking reduced AA formation and energy consumption but resulted in uneven colour and higher final water content. Secondary baking improved colour but did not fully resolve moisture issues, in the case of infrared baking. Sensory evaluation showed that texture and appearance strongly influenced acceptability.

Microwave baking did not affect the RS content of the biscuits. Since RS plays a key role in modulating the GI, the absence of an RS increase suggests that microwave baking is unlikely to produce a substantial reduction in eGI of the studied biscuits. For a more accurate evaluation of the biscuits' GI, *in vivo* measurements are recommended.

Substituting wheat flour with acorn or raw carob flour enhanced dietary fibre content and altered dough rheology and biscuit texture. Acceptable sensory quality was maintained for up to 20% substitution, with 20% acorn flour offering the best balance between nutrition, texture, and taste.

Energy consumption calculations revealed that microwave baking was generally more energy-efficient than conventional baking, although methodological differences suggest caution in direct comparison.

Analysis of gas release from LAs showed that much of the CO_2 escapes during baking, highlighting the challenges of achieving equivalent expansion without chemical leaveners.

5.2. Critical reflections on the PhD project

During this PhD project, several technical and practical challenges were encountered that affected both the progress and the overall results. While these challenges provided valuable experience and understanding of the biscuit baking process, they also highlighted the limits of what could be achieved under laboratory conditions.

- Process Optimisation and time constraints

A considerable amount of time was spent setting up and stabilising the reference process conditions, including flour mixing, dough preparation, and baking parameters. Although this step was essential to gain the necessary process know-how, it also led to time losses during the initial phases of the project.

- Limitations of laboratory-scale processing

Reproducing consistent results at a laboratory scale proved difficult. The small batch size and lack of precise control compared to industrial setups resulted in variability between trials. This made it challenging to apply the findings directly to industrial-scale biscuit production.

- Non-uniform microwave heating

Microwave processing showed limitations due to non-uniform energy distribution. Uneven heating affected texture development, leading to inconsistent product quality. Despite several optimisation attempts, this issue could not be fully resolved under the available lab conditions.

- Limitations in CO_2 Mixing

The incorporation of CO_2 into the dough matrix was another major challenge. The dough's ability to absorb and retain CO_2 was limited, even under 0.5 bar pressure. While acceptable results were obtained for softer baked products such as bread and cake, the approach was not effective for biscuit

dough. This limitation was mainly due to the dense structure and lower gas-holding capacity of biscuit formulations, combined with the restrictions of lab-scale equipment.

5.3. Perspectives and future works

- Improving Gas Incorporation

Future works could focus on developing systems that better represent real processing conditions. Using high-pressure CO_2 mixing equipment may help achieve higher gas solubility and more uniform distribution in doughs. This would improve aeration and texture in biscuit products.

- Continuous microwave baking systems

To address the problem of uneven heating, the use of continuous microwave baking systems is recommended. A novel design for biscuit baking can improve the biscuit baking procedure and reduce energy consumption. A final conventional baking step may still be required to complete drying and ensure a desirable biscuit texture.

- Product reformulation and ingredient alternatives

Future studies could also explore reformulation using different ingredients. One interesting option is roasted carob powder, which could be investigated as a chocolate replacer in biscuits. Its roasted flavour could provide appealing sensory qualities and new product opportunities.

- *In vivo* evalua*tion of GI*

An *in vivo* evaluation of the GI could be conducted to validate the nutritional properties of the product.

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7. PUBLICATIONS AND COMMUNICATIONS

7.1. Seminars and international mobility

- EIR-A (Agreenium) seminar in Bordeaux
  12-15 June 2023
- Poster at ICEF14 congress (**Alternative baking of dry cereal products considering microwaves and infrared to reduce the baking energy and to enhance the final quality of biscuits**)
  20-23 June 2023
- Oral presentation at CESM 2024 congress in Zagreb (**Alternative strategy to baking soda in biscuits thanks to mixing under CO_2 gas; impact on quality, including acrylamide level)**
  10-12 April 2024 (https://cespringmeeting.eu/book-of-abstracts/)
- Oral Presentation at ICEF 2024 congress in Nantes (**Biscuits free of baking soda thanks to mixing under CO_2; towards the next generation of biscuits**)
  22-25 April 2024
- EIR-A (Agreenium) seminar in Montpellier
  3-7 June 2024
- International mobility of EIR-A (Agreenium) in Greece
  3 months (2024-2025)

7.2. Publications

- **Microwave Baking of Biscuits; Towards the Next Generation of Biscuits with the ABCD Project**, IMPI 59th Annual Microwave Power Symposium, 2025 proceedings, ISSN 1070-0129.
- **Interest in biscuit dough mixing under CO_2 pressure as a strategy to reduce chemical leavening in laminated biscuits**, submitted to the *Journal of Food Engineering*.

Titre : Cuisson alternative des produits céréaliers secs

Mots clés : Biscuit laminé, Biscuit rotatif, Pétrissage au CO_2, Micro-ondes, Acrylamide

Résumé : Dans le cadre du projet ANR CAPS, cette thèse vise à développer des méthodes de cuisson économes en énergie (en utilisant les biscuits comme modèle) permettant d'améliorer la qualité nutritionnelle et de réduire la formation d'acrylamide (AA). La cuisson traditionnelle à haute température consomme beaucoup d'énergie et favorise la formation d'AA, un cancérigène probable, tout en affectant la gélatinisation de l'amidon, la texture et les propriétés sensorielles.

L'étude a exploré des recettes reformulées ainsi que des méthodes de cuisson alternatives (micro-ondes + infrarouge ou cuisson conventionnelle) afin d'améliorer la qualité nutritionnelle et de réduire l'impact environnemental. Deux types de biscuits (laminés et rotatifs) ont été utilisés pour : a) adapter les formulations (par exemple, le pétrissage au CO_2) afin de réduire les agents levants chimiques ; b) développer une cuisson au micro-ondes ; c) évaluer l'impact de la cuisson sur l'indice glycémique estimé (eIG) à l'aide de méthodes *in vitro* ; et d) améliorer la valeur nutritionnelle grâce à l'utilisation de farines de gland et de caroube. La cuisson au micro-ondes a nécessité une deuxième étape (infrarouge ou conventionnelle) et a produit des biscuits plus fermes et moins développés.

Le pétrissage au CO_2 a permis de réduire, mais pas d'éliminer, les agents levants sans compromettre la texture. L'AA a été réduit et l'eIG est resté inchangé. L'acceptation par les consommateurs a été bonne malgré une texture plus ferme.

Title: Alternative baking of dry cereal products

Keywords: Laminated biscuit, Rotary biscuit, CO_2 mixing, Microwaves, Acrylamide

Abstract: This thesis, as part of the ANR CAPS project, aims to develop energy-efficient baking methods (using biscuits as a model) that improve nutritional quality and reduce acrylamide (AA) formation. Traditional high-temperature baking consumes significant energy and promotes AA formation, a probable carcinogen, while also affecting starch gelatinisation, texture, and sensory properties.

This study explored reformulated recipes and alternative baking methods (microwave + infrared or conventional) to improve health and environmental impact. Two biscuit types—laminated and rotary—were used to: a) adapt formulations (e.g., CO_2 mixing) to reduce chemical leaveners;b)develop microwave-based baking; c) assess the impact of baking on the estimated glycaemic index (eGI) using *in vitro* methods; and d) improve nutritional value using acorn and carob flours. Microwave baking required a second stage (infrared or conventional) and produced firmer, less-developed biscuits.

CO_2 mixing helped reduce, but not eliminate, the leavening agents without compromising the texture. The AA level was reduced, while eGI remained unaffected. Consumer acceptance was good despite the firmer textures.