UNIVERSIDAD AUTÓNOMA DE MADRID

ESCUELA DE DOCTORADO

Development of novel functional dairy foods using byproducts from the coffee and wine industries for sustainable nutrition and health

Desarrollo de nuevos productos lácteos funcionales a partir de subproductos de la industria del café y el vino para una nutrición y salud sostenible

Maite Iriondo de Hond

Madrid, Febrero 2019

Eugenio José Miguel Casado, Dr. en Biología y Jefe de Servicio de Investigación Agroalimentaria del Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario (IMIDRA) y María Dolores del Castillo, Dra. en Ciencia y Tecnología de los Alimentos e Investigadora Científica de Instituto de Investigación en Ciencias de la Alimentación (CIAL) del Consejo Superior de Investigaciones Científicas (CSIC),

Informan:

Que el presente trabajo titulado "**Development of novel functional dairy foods using byproducts from the coffee and wine industries for sustainable nutrition and health**" (Desarrollo de nuevos productos lácteos funcionales a partir de subproductos de la industria del café y el vino para una nutrición y salud sostenible) constituye la memoria que presenta Maite Iriondo de Hond, Ingeniera Agrónoma por la UPM, para optar al GRADO DE DOCTORA. La presente memoria ha sido realizada bajo su dirección en el Departamento de Investigación Agroalimentaria del IMIDRA y el Departamento de Bioactividad y Análisis de Alimentos del CIAL (UAM-CSIC) y reúne las condiciones necesarias para su presentación y defensa.

Y para que conste a los efectos oportunos, firmamos el presente certificado,

Fdo.: Dr. Eugenio José Miguel Casado Fdo.: Dra. M.ª Dolores del Castillo Bilbao

En Madrid, Febrero de 2019

“*There is a place, like no place on earth.*

A land full of wonder, mystery, and danger.
Some say, to survive it, you need to be as mad as a hatter.
Which, luckily, I am”

Lewis Carroll, Alice in Wonderland

AKNOWLEDGEMENTS

> *Read the directions and directly you will be directed in the right direction*

Lewis Carroll, Alice in Wonderland

I guess that if a PhD was that easy, as to "*follow directions into the right direction*", we would miss half of the fun, learn half of what is there to learn, and we wouldn´t grow into our highest potential. And because it is simply not that easy, I know I wouldn't have been able to do it alone.

I want to start by thanking my PhD supervisors Eugenio José Miguel and Mª Dolores del Castillo for their guidance and support. Thank you for your advice, the opportunities you have given me, your enthusiasm and affection, but also for giving me the space to make my own mistakes and the opportunity to learn from them. I am grateful for the confidence you have had in me. I feel that I have become a more independent woman thanks to all these years learning from you. I have been very lucky to belong to two different families during this PhD, and I am taking the best from each one with me.

I am also grateful to my academic tutor, Tiziana Fornari, for helping me in every step of the way. To Félix Cabello, for believing in me from the beginning. I am honored for the opportunities you have given me. Thank you.

The exciting part of this thesis is that everything has been new. Coming from a plant-genetics background, every day has been a new learning opportunity. I want to thank some people that have dedicated their time to help me gain knowledge on their fields of expertise: Paula Varela, Carlos Oscar Sorzano, Malen Sarasua, etc. Thank you for your dedication and patience. I also want to thank Iván, Mario, Nasua and María from Vega de San Martín. Thank you for letting me be part of your team. I have enjoyed every minute with you. I already miss our experiments and the smell of cheese curd. I also want to thank Supracafé and Feiraco for giving me the opportunity of working in their projects.

Thank you, Pili, for being such a warrior and always looking out for me. Almudena, Nacho, Ana and Javier for your help and predisposition to do more. Also, to all the students (Jose Manuel, Jessica, Laura, Dani and Alba) that came to learn, and from whom I have learnt perhaps even more! My IMIDRA PhD fellows, Alberto, Alicia, Julia, Marga, Jose… if only walls could talk! "Dani garbanzos" for listening and listening and… did I mention listening? Zulima and Raul, you are wonderful. Thank you all for your friendship. And to sum up, to

all the IMIDRA family, we have made this journey together and each one of you has helped me in some way, even if it was just with a cup of coffee and a smile.

And then, someone reminded me that science is for society and encouraged me to "do my part". Thank you, Cristina de Lorenzo, for being such a wonderful inspiration and beautiful human being. That push gave birth to "AlimentUS", and I couldn't be happier for this amazing team of "science freaks". Bea, Nuri, Miguel, Moni, Tere, Adriana, Amaia… there is not much to say, you guys know you are the best.

I am so grateful to the CIAL family. I have been welcomed in this institution with open arms and I am humbled by its kindness. Thank you to the Bioscience group again, for being so welcoming. Thank you to Nerea and Diego, for sharing our Foodio experience; to Gorka, for the laughs; to my international friends Alessandro, Karla, Ixchel, Marco, Bruno; to "las Maites", to Jose, even though you support the wrong soccer team; and to the Vousse Team, for the hard work and exciting experience.

Thank you to everyone that came to the dairy product tastings and to the volunteers in the nutrition trial. This thesis has been possible thanks to you.

To my "Mafia" girls, thank you so much for your sparkling humor, and for your support when I needed it. Thank you, Mabi, Ana and Martolo, for your support, specially at the end. And to my "Agronautas", thank you. You are the best friends anyone could ask for. Inesita and Arantxa, thank you for being so close, even though you are so far away.

And to my family,

The greatest gift of my PhD has been sharing this experience with my little sister, who has grown to be a respectful and wonderful woman. I have enjoyed every minute we have worked together, our morning coffees at 7 am in an empty lab, our crazy chocolate (or polvorón) cravings and our PhD-travel adventures. Thank you Amaia for your help, support and infinite patience. I am going to miss the "Iriondo Team", but I'm sure we are still going to do great things together. I love you and I am very proud of you. (And I am also very proud of Andrés, who is finally in the right team!).

My greatest strength has come from your love, Dani. Thank you for being my best friend, and now, the best husband in the world too. And of course, I am most grateful to my parents. Thank you, Mommy, for your "simplifying-life lessons", because it sure made this journey a lot lighter. You always bring me down to earth, to what really is important. And thank you, Aita, for listening, guiding me and helping me not to lose my motivation. I admire you and love you both so much, I am the luckiest.

Now Kiwi… you are a PhD cat!

GRANTS AND FUNDING

The present study has been funded by the following projects:

1. Elaboración de nuevos productos lácteos funcionales ricos en compuestos antioxidantes (FP16-LACT) funded by IMIDRA.
2. Producción y consumo sostenibles del café: validación de subproductos como ingredientes alimentarios (SUSCOFFEE, AGL2014-57239-R) funded by the Ministry of Economy and Competitiveness.

Maite Iriondo de Hond received financial support with a PhD scholarship funded by the Ministry of Environment and Territory Planning of the Community of Madrid.

International mobility was funded by EIT Food as part of the FOODIO project.

LIST OF PUBLICATIONS

Research from this PhD Thesis resulted in the following publications:

Research articles:

- **Iriondo-DeHond, M.,** Miguel, E.; del Castillo, M.D., (2018), "Food Byproducts as Sustainable Ingredients for Innovative and Healthy Dairy Foods", *Nutrients*, 10, 1358.
- **Iriondo-DeHond, M.,** Blázquez-Duff, J.M., del Castillo, M.D., Miguel, E. (2019). Wine-making byproduct extracts and dietary fiber in the development of yogurts for sustainable health. *In preparation.*
- **Iriondo-DeHond, M.,** Varela, P., del Castillo, M.D., Miguel, E. (2019), The sustainability effect: consumers' perception on the use of byproducts from the wine industry as novel ingredients in yogurt. *In preparation.*
- **Iriondo-DeHond, M**., Iriondo-DeHond, A., Herrera, T., Miguel, E. & del Castillo, M.D., (2019). Comparative study of the sensory quality and health promoting properties of yogurts containing coffee and wine-making byproduct extracts. *In preparation.*
- **Iriondo-DeHond, M.,** Iriondo-DeHond, A., Herrera, T., Fernández-Fernández, A.M., Sorzano, C.O., Miguel, E. & del Castillo, M.D., (2019). A multidisciplinary approach for the optimization of the quality and health benefits of yogurts containing coffee cascara and inulin. *In preparation.*
- **Iriondo-DeHond, M**., Martín, A., Miguel, E., del Castillo, M.D., (2018), "In vitro determination of potential antioxidant and postprandial glucose response properties in yogurt formulations containing coffee byproducts", *Nutrición Clínica y Dietética Hospitalaria*, 38 (supl.1):72-73.

Book chapters:

- M.D. del Castillo, A. Iriondo-DeHond, N. Martinez-Saez, B. Fernandez-Gomez, **M. Iriondo-DeHond**, J.R. Zhou. (2017) "Applications of recovered compounds in food products", in: C. Galanakis (Ed.) *Handbook of Coffee Processing By-Products*. American Press, pp.171-194. ISBN 9780128112908
- **Iriondo-DeHond, M**., Miguel, E., del Castillo, M.D., (2019). "Byproducts as a Source of Novel Ingredients in Dairy Foods", in: Ferranti, P., Berry, E.M.,

Anderson, J.R. (Eds.) *Encyclopedia of Food Security and Sustainability*, Elsevier, vol. 1, pp. 316–322. ISBN: 9780128126875

Other publications related to the PhD Thesis include:

— Cruz-Maceín, J.L., **Iriondo-DeHond, M.,** Miguel, E., (2019), "Growth of artisanal cheese production, cheese culture, drivers and consumer profile: the case of the Community of Madrid", *In preparation.*

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ABBREVIATIONS

AECOSAN	Spanish Agency for Consumer Affairs, Food Safety and Nutrition
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	Analysis of variance
BMI	Body mass index
C3G	Cyanidin-3-O-glucoside chloride
CAF	Caffeine
CAP	Common Agricultural Policy
CAT	Catechin
CATA	Check all that apply
CGA	Chlorogenic acid
CFU	Colony-forming unit
CIAL	Instituto de Investigación en Ciencias de la Alimentación
CLA	Conjugated linoleic acid
CZE	Capillary zone electrophoresis
DHA	Docosahexaenoic Acid
DM	Diabetes mellitus
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DPPH	1,1-Diphenyl-2-picryl-hydrazy
DRI	Dietary reference intake
EC	European Council
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic Acid
EU	European Union
FA	Ferulic Acid
FAO	Food and Agriculture Organization of the United Nations
FBS	Fetal calf serum
FDA	Food and Drug Administration from the United States of America
FOS	Fructo-oligosaccharides
GAE	Gallic Acid Equivalent
GIP	Gastric inhibitory polypeptide

GLP-1	Glucagon-like peptide-1
GRAS	Generally recognized as safe
HACCP	Hazard Analysis and Critical Control Points
IC50	Half maximal inhibitory concentration
IEC-6	Intestine epithelial cells
IFT	Institute of Food Technologists
JAR	Just about right
LPS	Lipopolysaccharides
MRS	Man Rogosa Sharpe
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
MUFA	Monounsaturated fatty acids
NO	Nitric oxide
ORAC	Oxygen Radical Absorbance Capacity
PUFA	Polyunsaturated fatty acids
PYY	Peptide tyrosine tyrosine
ROS	Reactive oxygen species
SCFA	Short-chain fatty acids
SENC	Spanish Society of Community Nutrition
SFA	Saturated fatty acids
T2D	Type 2 diabetes
T1D	Type 1 diabetes
tBOOH	tert-butyl hydroperoxide
TPC	Total phenolic content
UHT	Ultra-high temperature
VAS	Visual analogue scale
VSM	Vega de San Martín
WHO	World Health Organization

SUMMARY/ RESUMEN

“ *Every adventure requires a first step* ”

- *The Cheshire Cat*

Lewis Carroll, Alice in Wonderland

SUMMARY

Providing a growing population with healthy diets from sustainable food systems is an immediate global challenge. In a context where food byproducts represent an environmental issue and diet-related chronic diseases are still projected to increase, we propose the development of novel functional dairy foods without added sugars, containing dietary fiber and bioactive compounds extracted from coffee and wine-making byproducts involved in the carbohydrate metabolism for the prevention of diet-related chronic diseases, such as diabetes and obesity.

The aim of the present PhD thesis was to evaluate the potential of using coffee (cascara and coffee silverskin) and wine-making (grape pomace, seeds and skins) byproducts as food ingredients for the development of innovative and sustainable functional yogurts. The new product development was conducted following a holistic approach to optimize the technological, biological, nutritional and sensory dimensions of yogurts. The selection of the coffee and wine-making byproducts was based on findings from a literature review conducted in the present thesis, results previously generated from our research group and those derived from the present investigation in relation to the *in vitro* antioxidant, antidiabetic and anti-inflammatory properties of the byproduct extracts.

A first prototype of yogurts containing wine-making byproducts was developed which showed that the *in vitro* antioxidant and antidiabetic properties of the grape pomace, seed and skin extracts were present after their addition into a yogurt matrix with dietary fiber (inulin and FOS), whose combination had not been previously studied for the development of functional yogurts. In addition, yogurts showed stable physicochemical, textural and microbiological characteristics during storage, assuring an optimal 21-day shelf life.

Consumer perception towards the use of food byproducts as novel ingredients in dairy foods was evaluated using the grape pomace, seed and skin yogurts. Overall liking was influenced by the type of byproduct, the evaluation condition and its interaction. Liking in expected (6.7) and informed (6.6) conditions was significantly ($p < 0.05$) higher than in blind conditions (6.3), suggesting that information regarding the origin of the ingredients improved the overall liking of the yogurts. In addition, consumers showed positive associations between the yogurts in informed conditions with non-sensory terms such as “healthy”, “antioxidant”, “high in fiber”, “satiety”, “sustainable” and “novel”.

The comparative study of the biological properties of yogurts containing coffee and wine-making byproduct extracts identified the coffee cascara yogurt as the most suitable

candidate as a potential functional food. Coffee cascara yogurt showed a high sensory acceptance (6.96), high antioxidant capacity (5.03 ± 0.11 µTE/g yogurt), the best inhibition of α-glucosidase activity (83%) and a significant ($p < 0.05$) reduction of NO levels at 10 mg/mL. In addition, the digest obtained from the simulated human digestion of coffee cascara yogurt showed significantly higher ($p < 0.05$) antioxidant capacity and greater inhibition of α-glucosidase than the control digest. The risk-benefit balance assessment of the cascara yogurt to adjust the concentration of dietary fiber (0, 3, 7 and 13% of inulin) avoiding potential gastrointestinal secondary effects was conducted in a blind crossover nutritional trial in healthy adults (n = 45) (Registration No. NCT03539146). Results showed that the cascara yogurt containing 3% of inulin was the most suitable yogurt formulation to contribute to the dietary recommended intake of fiber, as it could be labelled with the nutritional claim "source of fiber", without causing secondary gastrointestinal symptoms.

In conclusion, findings derived from the present PhD thesis confirmed the feasibility of using coffee and wine-making byproducts as sustainable ingredients in yogurt innovations. The application of the multidisciplinary approach proved to be an effective strategy for the development of safe and high quality products in terms of their physicochemical, sensory and health-promoting properties, which may contribute to commercialization and consumer adhesion to the product. The coffee cascara yogurt containing 3% inulin and no-added sugars may stand as a healthier and more sustainable alternative to current market options.

RESUMEN

En la actualidad, uno de los mayores retos a nivel mundial es proporcionar dietas saludables, a partir de sistemas de producción de alimentos sostenible, a una población en continuo crecimiento. Debido al gran problema medioambiental que generan los subproducts derivados de la industria alimentaria, y a la previsión de incremento en la incidencia de enfermedades crónicas no transmisibles, proponemos el desarrollo de nuevos productos lácteos funcionales sin azúcares añadidos, que contengan fibra dietética y compuestos bioactivos extraídos de subproductos del café y del vino, que participan en la regulación del metabolismo de los carbohidratos, para la prevención de enfermedades crónicas relacionadas con la dieta, como la diabetes y la obesidad.

El objetivo de la presente tesis doctoral es evaluar el uso potencial de subproductos de café (cáscara y cáscarilla de café) y del vino (orujo de uva, semillas y pieles) como ingredientes alimentarios para el desarrollo de yogures funcionales innovadores y sostenibles. El desarrollo del nuevo producto se realizó siguiendo un enfoque integral para optimizar las propiedades tecnológicas, biológicas, nutricionales y sensoriales de los yogures. La selección de los subproductos de café y de la producción del vino se basó en los resultados de una revisión de la literatura realizada en la presente tesis, en resultados generados previamente por nuestro grupo de investigación y en aquellos resultados derivados de la presente investigación con relación a las propiedades antioxidantes, antidiabéticas y antiinflamatorias obtenidas *in vitro* de los extractos de los subproductos de café y vino.

En primer lugar, se desarrolló un prototipo de yogures con extractos de subproductos vinícolas que mostraron que las propiedades antioxidantes y antidiabéticas *in vitro* del orujo de uva, y los extractos de semillas y la piel estaban presentes después de su adición en la matriz de yogur con fibra dietética (inulina y FOS), cuya combinación no había sido estudiada previamente para el desarrollo de yogures funcionales. Además, los yogures mostraron características fisicoquímicas, texturales y microbiológicas estables durante su almacenamiento en frío, asegurando una vida útil óptima de 21 días.

La percepción del consumidor sobre el uso de subproductos alimenticios como nuevos ingredientes en productos lácteos se evaluó utilizando yogures con orujo de uva, semillas y piel de uva. La aceptación general estuvo influenciada por el tipo de subproducto, la condición de evaluación y su interacción. La aceptación genral en las condiciones esperadas (6.7) e informadas (6.6) fue significativamente mayor ($p < 0.05$) que en las condiciones ciegas (6.3), lo que sugiere que la información sobre el origen de los ingredientes mejoró la percepción general de los yogures. Además, los consumidores

mostraron asociaciones positivas entre los yogures en condiciones informadas con términos no sensoriales como "saludable", "antioxidante", "alto en fibra", "saciedad", "sostenible" e "innovador".

El estudio comparativo de las propiedades biológicas de los yogures con extractos de subproductos de café y vino identificó al yogur de cáscara de café como el candidato más adecuado para su uso como alimento funcional. El yogurt de cascara de café mostró una alta aceptación sensorial (6,96), una alta capacidad antioxidante (5,03 ± 0,11 µTE / g de yogur), la mejor inhibición de la actividad de la α-glucosidasa (83%) y una reducción significativa ($p < 0.05$) de los niveles de NO en una concentración de 10 mg / mL. Además, el digerido del yogur cascara de café mostró una capacidad antioxidante significativamente mayor ($p < 0.05$) y una mayor inhibición de la α-glucosidasa que el digerido del yogur control. Se llevó a cabo una evaluación de la relación riesgo-beneficio del yogur de cáscara con el objetico de ajustar la concentración de fibra dietética (0, 3, 7 y 13% de inulina) para evitar los posibles efectos secundarios gastrointestinales. Para ello, se realizó en un estudio de intervención nutricional en humanos en condiciones ciegas, cruzadas y aleatorias con voluntarios adultos sanos (n = 45 (Número de registro NCT03539146). Los resultados mostraron que el yogur de cáscara que con un 3% de inulina fue la formulación de yogur más adecuada para contribuir a la ingesta diaria recomendada de fibra dietética sin causar síntomas gastrointestinales secundarios. El nuevo producto podría etiquetarse con la declaración nutricional “fuente de fibra”.

En conclusión, los resultados derivados de la presente tesis doctoral confirman la posibilidad de utilizar los subproductos derivados del café y el vino como nuevos ingredientes para el desarrollo de yogures funcionales saludables y sostenibles. La aplicación del enfoque multidisciplnar demostró ser una estrategia eficaz para el desarrollo de productos seguros y de alta calidad en cuanto a sus propiedades físicoquímicas, sensoriales y beneficiosas para la salud. Dicha optimización podría facilitar la comercialización y adhesión del consumidor a los nuevos productos desarrollados. El yogurt de cáscara de café con un 3% de inulina y sin azúcares añadidos representa una alternativa más saludable y sostenible a las opciones actuales del mercado.

INTRODUCTION

“*Begin at the beginning,” the King said, very gravely, “and go on till you come to the end: then stop.*”

Lewis Carroll, Alice in Wonderland,

1. A European vision for sustainability

The next steps for a sustainable European future include strengthening the connection between economy, society and the environment. Food systems have the potential to nurture human health and support environmental sustainability; however, they are currently threatening both [1]. Therefore, the 2030 European Bioeconomy Strategy and the Committee on Global Food Security from Food and Agriculture Organization of the United Nations (FAO) have set guidelines for the promotion of a global sustainable development. In this context, the following definitions are introduced:

FAO [3] defines Sustainable Diets as: "*those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources*".

According to FAO [4], Sustainable Food Systems are "*food systems that deliver food security and nutrition for all without compromising the economic, social and environmental bases to generate food security and nutrition for future generations*".

The European Food Safety Authority (EFSA) [5] defines Functional Foods as: "*A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. A functional food can be a natural food or a food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet*".

Sustainable Health is defined as: "*a healthy and active ageing avoiding the risk of diseases"* [2].

The provision of healthy diets to the growing population is now contemplated from a sustainability perspective. This is necessary in a context where the prevalence of diet-related chronic diseases, such as obesity and diabetes mellitus (DM), has become one of the biggest global health crises of the 21st century.

Diet-related chronic diseases pose a substantial health and economic burden, which cause 17 million deaths worldwide [6], and have been estimated to cause $17.3 trillion of cumulative economic loss between 2011 and 2030 [7]. Obesity is a chronic disease

characterized by the expansion of adipose tissue and an inflammatory component [8]. DM is a multifactorial disease characterized by persistent elevated blood glucose, which is caused by either pancreatic failure to secrete insulin (type 1 diabetes; T1D) or cellular failure to respond properly to insulin, a condition known as insulin resistance (type 2 diabetes; T2D) [9]. Several epidemiological studies have described a parallel escalation of obesity and DM. The connection between obesity and diabetes is attributed to insulin resistance and insulin deficiency [10].

In Spain, the ALADINO Study indicated that the prevalence of overweight and obesity among children between 6 and 9 years old are among the highest in Europe [11]. In the adult population, the prevalence of overweight in 2017 was 37%, whereas obesity prevalence was 17% [12]. Diabetes prevalence is 13.8% in the adult population, whereas 30% presents either impaired fasting glucose or impaired glucose tolerance [13].

The etiology of these metabolic chronic diseases has multiple determining factors including lifestyle and behavioral habits, which mean that it is possible to reduce the risk of these metabolic diseases through different prevention strategies. Diet quality plays a vital role in helping people with T2D to achieve and maintain optimal glycemic control, thereby lowering their risk of developing diabetes-related complications [14].

2. Dairy innovation: an opportunity for novel ingredients in functional foods

2.1. Market trends in the Spanish dairy sector

Dairy products stand as an essential component of the European diet. The Spanish Society of Community Nutrition (SENC) recommends a dairy product intake between 2 to 3 portions per day (Figure 1). The structural surplus in European milk production between 1970 and 1980 led to the implementation of the milk quota regime, which was introduced in 1984 in the EU by the Common Agricultural Policy (CAP) to control milk production in the different Member States, stabilize producer prices and maintain dairy activities in less competitive regions [15]. However, in April 2015 the EU milk quota regime came to an end due to the increasing milk and dairy demand and the need to encourage producers to become more competitive and market-oriented [16]. This scenario has brought the European dairy sector into a period of decreasing milk prices and increasing production of milk [17].

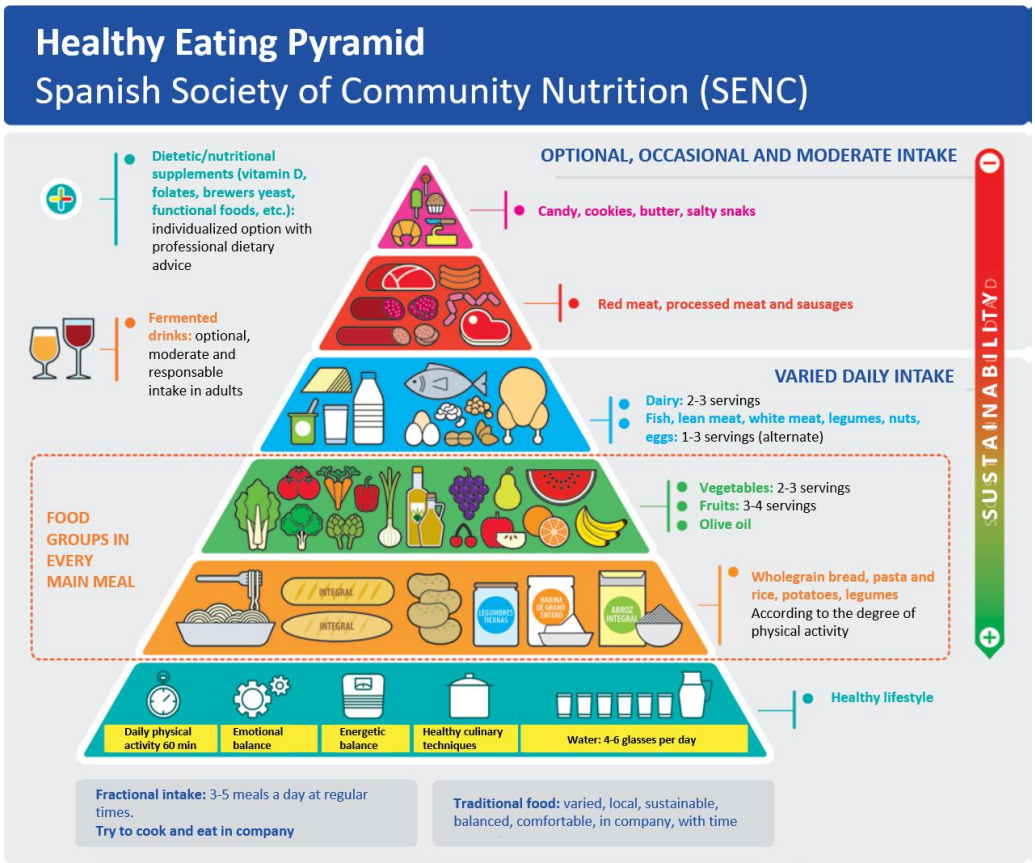

Figure 1. Healthy Eating Pyramid designed by the Spanish Society of Community Nutrition (SENC, 2018).

Within Europe, Spain stands as the 7th producer of cow's milk, accounting for 4% of the total European production. In relation to ewe's and goat's milk, Spain is the 1st and 2nd producer, accounting for 17% and 22% of the total production, respectively [18]. In the last years, milk and milk product consumption's trend is continuously decreasing (Figure 2). Milk prices are also dropping, with a greater price reduction in liquid milk (-1.9%) than in other milk products (-0.9%). At the same time, the ANIBES study reported that there is an important percentage of the Spanish population not meeting the recommended intakes for calcium, magnesium and vitamin D [19]. Therefore, incentives such as the

"Say yes to dairy, 3 portions per day" program from INLAC have been launched to promote dairy product consumption, which are excellent sources of these nutrients.

2017 Spanish Dairy Sector

MILK

Volume (1000 L): 3,187,960.1
Mean price (€/L): 0.69 (-1.9%*)

MILK PRODUCTS

Volume (1000 Kg - L): 1,749,483.98
Mean price (€/L): 3.28 (-0.9%*)

	Consumption (kg-L/person/year)	Consumption variation (%)*
Milk	69.9	4.2
Milk products	38.4	-3.1
Fermented milks	14.5	-6
Butter	0.3	0
Cheese	7.7	-4.7
Ice-cream and cakes	3.5	-3.1
Cream	1.0	-6.1
Desserts	6.09	-4.3
Milkshakes	3.96	0.3

*Per capita consumption and prize variation between 2016 – 2017.

Figure 2. Milk and milk product consumption volume, per capita consumption and mean prize in Spain in 2017 and its variation compared to 2016. Data collected from the 2017 Spanish Food Consumption Report [20].

In this context, product innovation is becoming increasingly important for the dairy industry as a means of improving profit margins. Health and sustainability-oriented consumer trends, such as the demand for functional and 'clean label' products, have been added to the traditional drivers of price, taste and convenience [21]. The dairy sector is well positioned to capitalize on this trend, as dairy products account for the largest share in the functional food market [22]. Being able to attach scientifically proven nutritional and health claims to products is a key element in increasing profit margins in this market [21].

Functional foods generally contain one or more beneficial compounds such as vitamins, minerals, fatty acids, prebiotics, probiotics, antioxidant polyphenols and sterols, carotenoids, etc. [23]. These bioactive compounds are considered the backbone of functional foods and are responsible for their health properties by preventing or managing chronic disease or its symptoms [24].

There are currently two types of claims that can be used on food labels to communicate health benefits: nutritional and health claims. These claims fall under the European Regulation (EC) No. 1924/2006 whose goal is to ensure a high level of consumer protection, give the consumer the necessary information to make choices in full knowledge of the facts, as well as creating equal conditions of competition for the food industry [5]. According to (EC) No. 1924/2006:

- Nutrition claims state, suggests or implies that a food has beneficial nutritional properties due to: 1) the energy it provides, provides at a reduced or increased rate or does not provide; and 2) the nutrients or compounds it contains, contains in reduced or increased proportions or does not contain. Common nutrition claims in dairy products include “fat free”, “low fat”, “sugar free”, “low sugar” and “low sodium”. More recently, the claims “source of protein” and “high protein” have been used in yogurts and cheese, as well as “source of fiber” in milk.
- Health claims state a relationship between food and health. These include: 1) “Function Health Claims” relating to the growth, development and functions of the body, to psychological and behavioral functions, or to slimming/ weight-control; 2) “Risk Reduction Claims”; and 3) “Claims referring to children's development”. Yogurt has its own approved health claim in Europe. Based on several human studies, the EFSA stated that a causal relationship exists between the consumption of live yogurt cultures in yogurt and improved lactose digestion in individuals with lactose maldigestion [25]. In addition, fermented milks containing plant sterols and stanols have been authorized the health claim “contributes to the maintenance of normal blood cholesterol levels” when consuming a minimum daily intake of 0.8 g of plant sterols/stanols [26].

2.2. Dairy reformulation policy for reducing diet-related chronic diseases

Although dairy products have a prominent position within the functional food market, government institutions have alerted about the excessive amount of added sugars and saturated fats found in many dairy products [27,28]. The Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) and other European institutions have set different guidelines for product reformulation, which aim to reduce the content of critical nutrients (saturated fats, trans fats, salt and sugar) in foods and beverages without involving an increase in the energy content or quantity of other nutrients of concern, and maintaining food safety levels and organoleptic properties so that the product continues to be accepted by the consumer. These guidelines are directed at all the actors involved in the food supply chain, including producers, processors and retailers.

Specific measures for dairy product reformulation (Figure 3) comprise a 10% reduction of the median content of added sugar in sweetened yogurt and fermented milks, reductions in the content of added sugars in dairy desserts (custards, puddings, etc.), reducing the size of the portions offered and raising public awareness about nutrients and their relation to health [29]. These product reformulation policies manifest the need to make healthier choices accessible and preferable in order to reduce the incidence of diet-related chronic diseases, such as obesity and T2D. In this sense, long term sugar reduction in dairy products may contribute to promoting a better dietary environment for diabetic patients.

Besides the sugar content reduction strategy, we propose implementing additional measures that may help in the design of reduced sugar products, based on the principles of combination therapy for diabetes prevention [30], which are: the use of dietary fiber, inhibitors of the carbohydrate metabolism, and their combination. This approach takes into consideration the dairy food product as a unit for the prevention of diet-related chronic diseases. This is, a food product that includes different ingredients with different mechanisms of action that may act synergistically in the prevention of the target diseases. The potential of using these ingredients in the development of healthier dairy products and the implications of sugar reduction in the sensory properties of the products are further discussed in sections 2.3. Dairy products for sustainable nutrition and health, and 2.4. The sensory challenge, respectively.

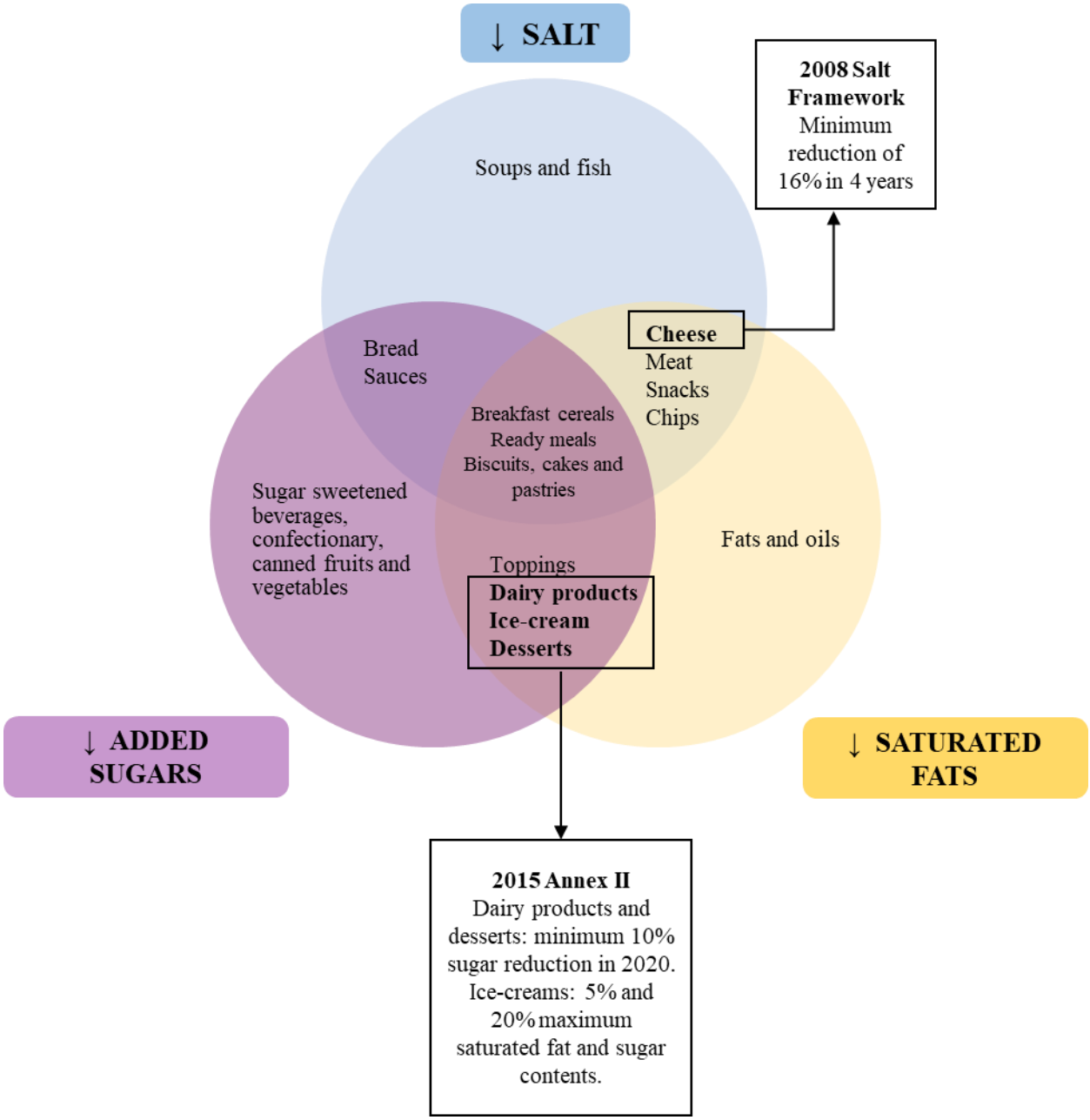

Figure 3. Priority food group products and food sectors for reformulating according to the frameworks and annexes of the High Level Group on Nutrition & Physical Activity. Figure from the Collaboration Plan for the improvement of the composition of food and beverages and other measures (AECOSAN) [27].

2.3. Dairy products for sustainable nutrition and health

Within the dairy section, the need for innovation brings the opportunity to use novel sustainable ingredients in the healthy-product reformulation scheme. The use of polyphenol rich food byproducts stands as a strategy for incorporating bioactive compounds in the human diet while reducing their environmental impact. In this sense, coffee and wine-making byproducts are currently receiving much attention because of their beneficial health effects related to their phytochemical content.

Coffee and wine-making byproducts

In 2016, more than 9 and 77 million metric tons of green coffee beans and grapes were produced, respectively [31]. The conversion of the coffee and grape berries into the popular beverages is responsible for the generation of large amounts of byproducts which represent an environmental issue. The anatomic structure of coffee and grape berries is shown in Figure 4.

Grape vines are mostly cultivated in developed countries. Spain is the third largest global wine-producer, with a production volume of 32.1 million hectoliters in 2017 [32]. Wine-making byproducts account for about 20-30% of the weight of the grapes used to make wine [33]. Therefore, considering the large wine production in Spain and its subsequent byproduct generation, the valorization of wine-making byproducts has a great economic and environmental interest for the Spanish agri-food industry.

Residues generated from the white or red wine production are partly different. In both cases, vine shoots are the first byproduct generated, resulting from in-field cultivation interventions, followed by the generation of grape stalks. Grape pomace is the main fraction of the solid wastes generated during wine-making processing, accounting for 60% of their weight, and consists of grape skins, seeds and residual stalks [34]. Grape pomace is immediately discarded in white wine production, whereas in red wines it is left with the juice for a fraction of the fermentation period. In this process, first the grape pomace is removed, followed by the wine lees. Grape processing also generates wastewaters and spent filter cakes.

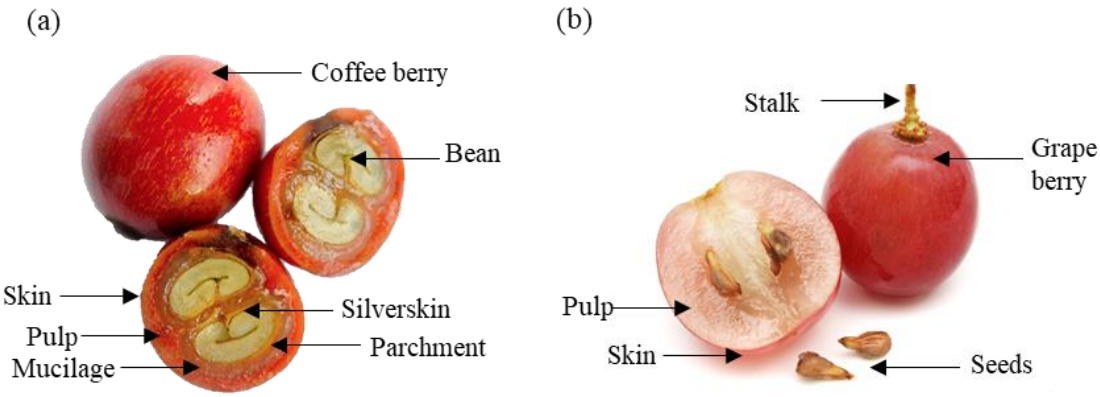

Figure 4. Cross-section of the (a) coffee cherry and (b) grape berry indicating their anatomic parts.

On the other hand, coffee is cultivated in developing countries, with Brazil, Vietnam, Colombia and Indonesia standing as the main producers [35]. Europe is the largest coffee consumer, with 45.3 thousand 60-kg bags consumed during 2018, and accounts for nearly 45% of the world's coffee bean imports [36]. Spain is the only European country where coffee is cultivated. The coffee plantation located in Agaete, in the Canary Islands, has a small production specialized in high quality coffee. Therefore, Spanish coffee consumption depends on green coffee beans imports that are processed by roasting companies.

Approximately 90% of the edible parts of the coffee cherry are discarded during its conversion into the coffee beverage [37]. Coffee is internationally traded as green coffee, which is produced either in the wet or dry processing. These processing methods generate slightly different byproduct fractions. Green coffee beans obtained via the wet processing method generate the skin and pulp in one fraction (also known as coffee cascara or coffee pulp), mucilage in a second fraction and finally the parchment [38]. The dry method generates a single byproduct fraction composed of the skin, pulp, mucilage and parchment all together, commonly known as coffee husks [39]. These byproducts are generated in coffee producing countries. Roasting of the green coffee beans is carried out in coffee consuming countries to prevent flavor and friability deterioration that could be caused by international circulation [40]. Thus, coffee consuming countries produce vast amounts of coffee silverskin, the only byproduct

resulting from the roasting of the green coffee beans, and spent coffee grounds, which arises from the production of instant coffee and coffee brewing [41].

In this sense, European coffee-roasting and consuming countries must deal with vast amounts of coffee silverskin and spent coffee grounds. Previous studies from our research group applied these byproducts as food ingredients in the development of novel beverages [42] and bakery products [43,44]. However, coffee cascara represents a greater environmental issue, as its generation is the largest compared to the rest of the coffee byproducts (it represents 29% of the dry weight of the coffee cherry). Therefore, more efforts are needed to valorize and find novel applications for coffee cascara.

Novel Food regulation for coffee and wine-making byproducts

A relevant consideration regarding the use of byproduct as food ingredients is their regulatory status (Figure 5). In December 2016 the EFSA included coffee cherries in the Novel Food Catalog [45]. This means that coffee byproducts husks and cascara need to undergo EFSA's authorization procedure according to regulation (EU) 2015/2283 before they can be introduced into the European food market. In this context, studies on the safety of coffee byproducts are needed to facilitate its permission. Previous research from our laboratory conducted the analyses of contaminants, including pesticides, mycotoxins, acrylamide, as well as acute toxicity experiments to validate the safety of using coffee husk, parchment and silverskin as safe ingredients [46].

Meanwhile, coffee cascara is an officially authorized ingredient in other parts of the world, such as Mexico or the US, where cascara has the generally recognized as safe (GRAS) status. Cascara beverages have become one of the latest product innovations launched by small businesses and multinational corporations, such as Starbucks [47].

On the other hand, wine-making byproducts derive from grapes that have been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force. Therefore, commercialization of grape pomace, seed and skin extracts is currently authorized in Europe and worldwide. However, grape seed meal, the residual from the grape seeds after oil has been extracted, requires authorization under Regulation (EC) 2015/2283.

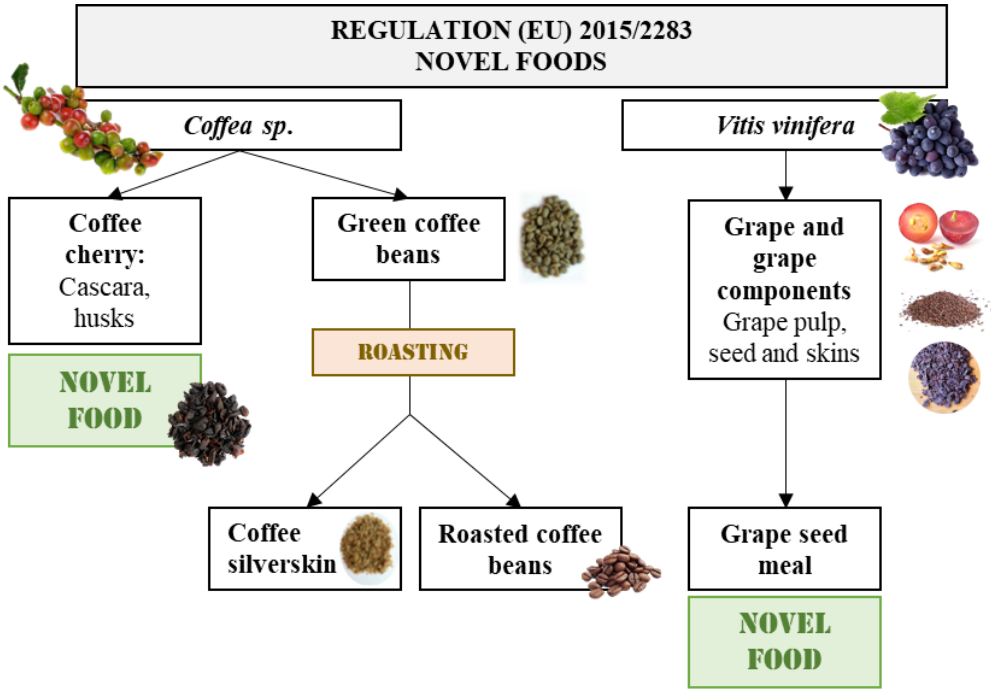

Figure 5. Novel food classification of coffee, grapes and their byproducts.

Use of food byproducts and dietary fiber in dairy product development

Several phenolic compounds have been described to have potential effects against hyperglycemia induced chronic diseases. The antihyperglycemic effects of polyphenols have been attributed to many biological properties including reduction in intestinal absorption of dietary carbohydrates, modulation of the activities of enzymes involved in glucose metabolism, protection of β cell from oxidative injury, and stimulation of insulin secretion and action [48]. Grape seed procyanidins and monomeric flavonols, monomeric flavanols and proanthocyanidins from grape skin extracts have been described to participate in the regulation of carbohydrate metabolism by inhibiting the activity of α-glucosidase enzyme [49,50]. Alpha-glucosidase is a membrane-bound enzyme located in the small intestine, which facilitates the absorption of digestible carbohydrates by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides [51]. Chlorogenic acid found in coffee and coffee silverskin byproduct also presented inhibition of the activity of α-glucosidase enzyme [52].

The EFSA Regulation (EU) 1169/2011 defines dietary fiber as: "*carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:(I) edible carbohydrate polymers naturally occurring in the food as consumed, (II) edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence, or (III) edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence"*

Inulin and fructo-oligosaccharides (FOS) are soluble, highly fermentable, non-digestible carbohydrates with low viscosity and low gel-forming properties [53]. Their difference lies on their degree of polymerization (DP) (Orafti®GR-FOS DP between 2 and 8; Orafti®P95-inulin DP between 2 and 60). The short-chain FOS is much more soluble and sweeter than long chain inulin, and is frequently used as a sugar replacer [54]. Long-chain inulin can act in rheological and sensory properties of dairy products as a fat substitute [55]. Their potential role in the prevention of diet-related chronic diseases is linked to the production of short-chain fatty acids (SCFAs) from their fermentation by colonic bacteria, which can contribute to regulating the secretion of gastrointestinal hormones involved in satiety and appetite control, such as glucagon-like peptide-1 (GLP-1) and ghrelin [56–58]. These effects have been described in *in vivo* studies in animal models [59] and human intervention trials [60]. Repeated consumption of inulin containing yogurts have been previously described to reduce subjective appetite ratings [61]. In addition, the ANIBES study has demonstrated an insufficient dietary fiber intake among the Spanish adult population [62], which is far below the EFSA recommended intake of 25 g of dietary fiber per day. Therefore, the incorporation of FOS and inulin in highly consumed dairy products, such as yogurts, may contribute to achieving the dietary fiber daily recommendations.

Current market options for dairy products, such as yogurts and desserts, include products with a high sugar content or their equivalent low sugar versions in which sucrose is partially replaced by sweetening agents. However, there is a lack of products that combine a high fiber content, absence of added sugars and incorporation of bioactive compounds involved in the regulation of the carbohydrate metabolism. This way, dairy products developed for sustainable health may have the potential to become a new product section, with a niche market targeted at health and environmentally conscious consumers.

2.4. The sensory challenge

Although the perceived importance of food for health is still an increasing trend, consumer's critical attitude towards functional foods is also increasing. This translates into lower willingness to compromise on taste for health [63]. Therefore, more effort is needed to develop healthy foods that satisfy the sensory demands of consumers.

In this context, policies for dairy product reformulation have given food industries a new challenge: to reduce sugar levels in their products without affecting their organoleptic properties, which are determinant for product success in the market [64]. Thus, food industries must be able to reformulate products so that they taste just as good as the original versions, solving the flavor and texture changes associated to sugar reduction. In this sense, inulin and fructo-oligosaccharides (FOS) have been described to improve the sensory acceptance of dairy products due to their technological properties [65]. Inulin has shown to be particularly suitable as a fat replacer and texture modifier, contributing to an overall improved mouthfeel [55]. On the other hand, FOS, which are produced by partial hydrolysis of inulin, are sweeter and are preferably used as sugar replacers [66].

During new product development, consumer acceptance is usually evaluated using hedonic tests which measure the overall liking of the product via the 9-point hedonic scale developed by Peryam and Pilgrim in 1957 [67]. Information about consumer's perception of the sensory characteristics of the products is often collected together with overall liking scores to identify prototypes that best align to consumer preferences and to obtain directions for product reformulation and improvement [68,69]. In this sense, Just-about-right (JAR) scales are a common approach to identify the optimal intensity of sensory attributes [70]. Check-all-that-apply (CATA) questions are currently a popular approach for getting sensory characterizations of foods by consumers as an alternative to the classical descriptive analysis by trained panels. Consumer-based approaches have been described to be more cost-efficient and less time consuming than conventional descriptive analysis, obtaining accurate product descriptions when using a high number of consumers (preferably over 100 consumers) [71].

Overall, it is relevant to develop functional foods from a holistic approach that includes experts from the Food Science field but also from Nutrition, Chemistry, Sensory and Consumer Science. The multidisciplinary approach for new product development should combine academic and industry sectors to respond to the good-tasting functional food demand.

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OBJECTIVES AND WORK PLAN

“ *One day Alice came to a fork in the road and saw a Cheshire cat in a tree.*
‘Which road do I take?’ she asked.
‘Where do you want to go?’ was his response.
‘I don’t know,’ Alice answered.
‘Then,’ said the cat, ‘it doesn’t matter. ”

Lewis Carroll, Alice in Wonderland

In the context of dairy product reformulation for sustainable health, yogurt formulations without added sugars, containing dietary fiber and bioactive components involved in the regulation of the carbohydrate metabolism, consumed as part of a healthy diet, could help reduce the risk of developing chronic disorders. We hypothesize that the combined use of coffee or wine byproduct extracts together with soluble non-digestible carbohydrates (inulin and/or FOS) could lead to yogurt formulations with a high nutritional value and an adequate sensory quality to satisfy the demands of consumers. The optimization of the formulations requires a multidisciplinary approach that considers the physicochemical, biological, nutritional and sensory dimensions of new product developments.

Based on this scenario, the **general objectives** for the present thesis were proposed:

1. To evaluate the potential of using coffee and wine-making byproducts as food ingredients for the development of innovative, healthy and sustainable yogurts.
2. To provide new knowledge on the physicochemical, biological, nutritional and sensory dimensions of yogurts containing coffee and wine-making byproducts.

In order to achieve this purpose, the following **specific objectives** were defined:

1. **Identification and selection of the food byproducts to be used as novel ingredients in the development of healthier dairy foods** *(Chapter 1)*.
   Chapter 1 provides a literature review on the applications of food byproducts used up to date on the development of novel dairy foods. Conclusions from this chapter helped in the selection of the byproducts and the dairy matrix used in the present thesis.

2. **Holistic quality assessment of yogurts containing wine-making byproducts** *(Chapter 2)*.
   A primary product formulation was proposed using wine-making byproduct extracts and dietary fiber (inulin and FOS) as ingredients in yogurt. The quality of yogurts containing wine-making byproducts was assessed in terms of its physicochemical, biological and sensory components (*Study 1*). Physicochemical parameters, nutrient composition, microbiological and textural parameters were analysed. As these parameters can experience changes along time, their stability was monitored during the product's shelf life. The *in vitro* antioxidant and antidiabetic properties of the byproduct ingredients and yogurts were evaluated as a first approach towards determining its potential in the regulation of the metabolism of carbohydrates for diabetes management and

prevention. A pilot sensory test was conducted to evaluate consumer's product acceptance.

The evaluation of the consumer's perception on the use of wine-making byproducts as ingredients in sustainable yogurts was conducted (*Study 2*). This study gave further insight into the marketability of yogurts containing wine-making byproducts.

3. **Comparative evaluation of the biological and sensory properties of yogurts containing coffee and wine-making byproducts** *(Chapter 3).*
   The health promoting properties of yogurts containing coffee byproducts and inulin were analyzed and compared to those of yogurts containing wine-making byproducts and inulin. The analyses included *in vitro* determination of the antioxidant, antidiabetic and anti-inflammatory properties and their maintenance after *in vitro* human digestion. A consumer test was performed to assess the yogurt's overall acceptability and its sensory attributes. The aim of the comparative study was to select a yogurt prototype with potential properties for diabetes management and prevention.

4. **Nutritional risk-benefit balance assessment of yogurts containing coffee cascara extract and dietary fiber** *(Chapter 4).*
   The yogurt formulation was finally optimized by adjusting the concentration of inulin according to its gastrointestinal tolerance. For this purpose, a crossover nutritional intervention study was conducted in healthy adults (Registration No: NCT03539146).

The main contributions presented in this thesis refer to the development of functional yogurts. In addition, the multidisciplinary approach combining the evaluation of technological, biological and sensory properties was also applied in the development of a) a novel cheese containing wine-making byproducts (*Annex I*), b) alternative plant-based desserts (*Annex 2*) and c) yogurts for the prevention of metabolic syndrome (*Annex 3*). These product developments have been carried out in collaboration with industrial partners.

The work plan followed to achieve the main and specific objectives defined in this thesis is presented in Figure 1.

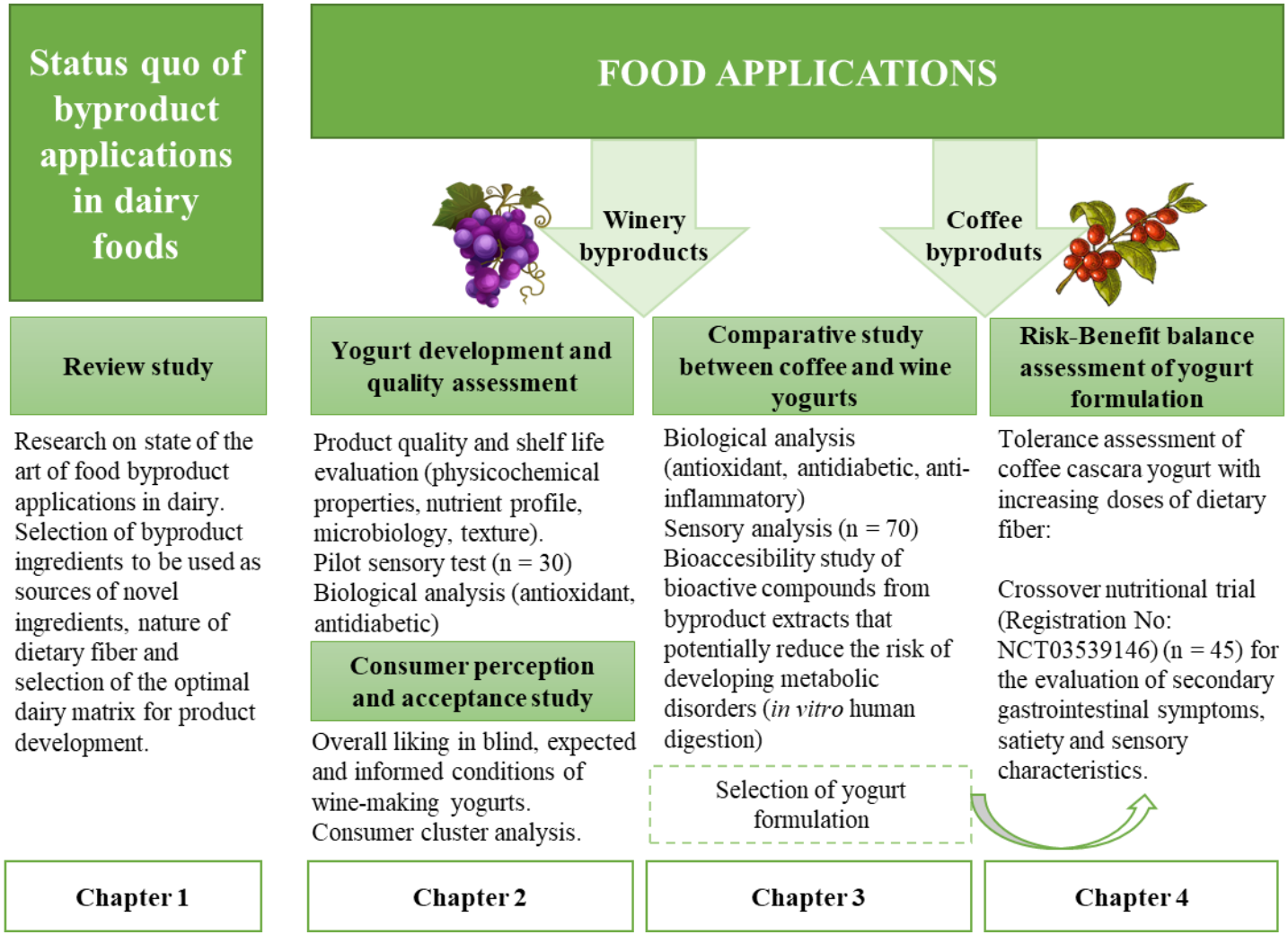


Figure 1. Schematic view of the workplan of the present study.

MAIN CONTRIBUTIONS

“*Everything's got a moral, if only you can find it.*”

- *The Duchess*

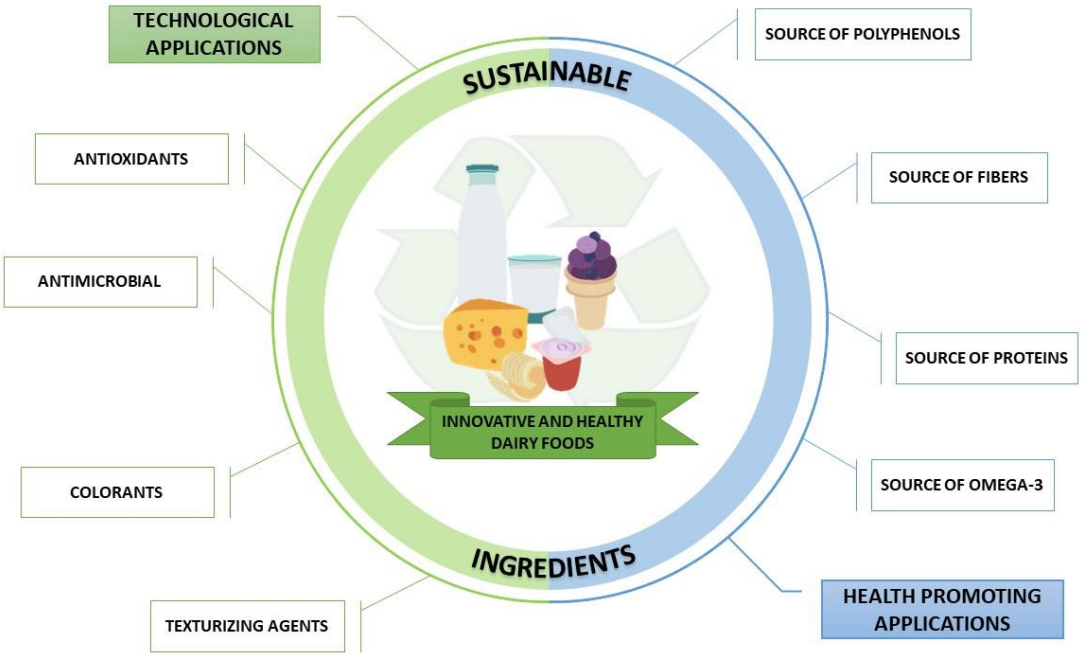
Lewis Carroll, Alice in Wonderland

CHAPTER 1

Food byproducts as sustainable ingredients for innovative and healthy dairy foods

This chapter provides a narrative review on the different applications of food byproducts used as ingredients in the development of dairy foods. Results from this chapter have been published in:

Iriondo-DeHond, M., Miguel, E. & del Castillo, M.D., 2018. Food Byproducts as Sustainable Ingredients for Innovative and Healthy Dairy Foods. *Nutrients*, 10(10), p.1358. Available at: http://dx.doi.org/10.3390/nu10101358.



Food Byproducts as Sustainable Ingredients for Innovative and Healthy Dairy Foods

Maite Iriondo-DeHond [1,2], Eugenio Miguel [1], María Dolores del Castillo [2]

1 Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), N-II km 38,200, 28800 Alcalá de Henares, Spain.

2 Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), C/ Nicolás Cabrera, 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

Nutrients 2018, 10, 1358. https://doi.org/10.3390/nu10101358

Abstract

The valorization of food wastes and byproducts has become a major subject of research to improve the sustainability of the food chain. This narrative review provides an overview of the current trends in the use of food byproducts in the development of dairy foods. We revised the latest data on food loss generation, the group of byproducts most used as ingredients in dairy product development, and their function within the food matrix. We also address the challenges associated with the sensory properties of the new products including ingredients obtained from byproducts, and consumers' attitudes towards these sustainable novel dairy foods. Overall, 50 studies supported the tremendous potential of the application of food byproducts (mainly those from plant-origin) in dairy foods as ingredients. There are promising results for their utilization as food additives for technological purposes, and as sources of bioactive compounds to enhance the health-promoting properties of dairy products. However, food technologists, nutritionists and sensory scientists should work together to face the challenge of improving the palatability and consumer acceptance of these novel and sustainable dairy foods.

Keywords: byproducts; sustainability; functional foods; dairy products

1. Introduction

Sustainability presents both an opportunity and a challenge to the dairy sector. It is an opportunity, because the possibility of using food-processing byproducts for bioactive compound and nutrient extraction has created enormous scope for waste reduction and indirect income generation [1]. However, the challenge is to sustainably intensify the global food production system to enhance food security and nutrition without sacrificing the environment, and to render the concept of sustainable functional foods into a marketable product that is acceptable to consumers [2,3].

The development of novel food and/or functional food products is increasingly challenging, as it has to fulfill the consumer's expectations for products that are simultaneously palatable and healthy [4]. Compared to conventional foods, the development of functional components and technological solutions can be demanding and expensive, and needs of a tight strategy between research and business. All this occurs in a context where functional food markets are continuously changing [5,6].

The purpose of this review is to summarize the research findings on the application of various food-processing byproducts used as a source of targeted compounds or as whole ingredients in the manufacturing of dairy foods. So far, most studies available on the valorization of agro-industrial food wastes focus on specific byproducts and their applications in different foods. In this review, the focus is on dairy product development, and how byproducts can be used in their manufacturing to improve their technological and health-promoting properties.

2. Materials and Methods

The present narrative review was conducted by a literature search consulting the PubMed, Web of Science and Scopus databases. The search was limited to English written articles published during the last 18 years, from January 2000 to July 2018. Search terms for general and specific food processing byproducts ("food byproduct", "food waste", "food loss", "vegetable byproduct", "fruit byproduct", "grape pomace", "orange pomace", "coffee byproduct", "cheese whey", "fish byproduct", "meat byproduct"), were combined with search terms for dairy matrices ("dairy", "yogurt", "fermented milk", "milk", "cheese", "butter", "ice-cream"). In addition, references of relevant reviews and original research articles were manually searched to find out more potential eligible studies. Data on legislation were consulted from the Codex Alimentarius guidelines, the United Nations Food and Agriculture Organization (FAO), the European Food Safety Authority (EFSA) and the Food and Drug Administration

(FDA). Data from the FAO Food Balance Sheets regarding worldwide production and losses of the different food commodity groups for the most recent year available (2013) were accessed to study the latest state of global food loss generation.

The selection of the papers to be included in the review was performed after a thorough study of their content by the authors. The information extracted from the identified references included first author's name, author affiliation, publication year, dairy product developed, food byproduct used as an ingredient, purpose of adding the food byproduct as an ingredient (technological or health-promoting function) and outcomes. The selection process resulted in the identification of 50 eligible studies which directly addressed the application of a food byproduct as an ingredient in a dairy matrix.

3. Byproducts Used as Novel Ingredients in Dairy Foods

Food loss was redefined by FAO in 2014 as "the decrease in quantity and quality of food". Food waste is considered as part of food loss and refers to discarding or alternative non-food use of food that is safe and nutritious for human consumption along the food chain [7]. Food losses and waste represent an imbalance in the availability and accessibility dimensions in the global food system. Different multifaceted strategies have been proposed by the FAO Committee on Global Food Security to promote the development of a sustainable food system, including food byproduct valorization. In this sense, a reduction in food losses and waste could potentially lead to positive economic, social and environmental outcomes, improving food availability and accessibility, and enhancing a sustainable use of natural resources on which the future production of food depends [8].

The most recent Food Balance Sheets [9] indicate that fruits and vegetables presented the highest values of food losses along the food chain compared to the rest of the commodity groups: cereals, roots and tubers, oilseeds and pulses, meat, fish and seafood, and dairy products. Correspondingly, there has been increasing interest in using fruit and vegetable byproducts as novel ingredients in the development of foods, including dairy products. This focus may be explained by several factors: their impact on the environment, their potential health-promoting phytochemical content, and the fact that plant-derived byproducts and losses mostly occur before household consumption, which makes them still available for reutilization.

Of the studies on the development of innovative and health-promoting dairy products using sustainable ingredients published from 2000 to 2018 (n = 50 eligible studies), 88% used side-streams from plant materials. Most studies used byproducts from fruits (43%),

followed by the application of winery (19%) and vegetable (13%) byproducts. Among fruit and vegetable byproducts, most research has been carried out using citrus and tomato side-streams as ingredients in dairy formulations (Figure 1), which means that efforts have been made to valorize byproducts from food groups that present some of the largest food losses [9]. In 2013 alone, 13.4 and 6.9 million oranges and tomatoes were lost during storage and transportation [9]. It is evident that the amount of food loss is correlated to the amount of the food item produced, but the ratio of food loss within a production chain for a specific item can also help identify which foods are more susceptible to being lost. As seen in Figure 2, bananas, plantains and pineapples have some of the highest loss rates among fruits and vegetables during storage and transportation. This way, further strategies for food loss and waste reduction could focus on using byproducts from these foods as novel ingredients.

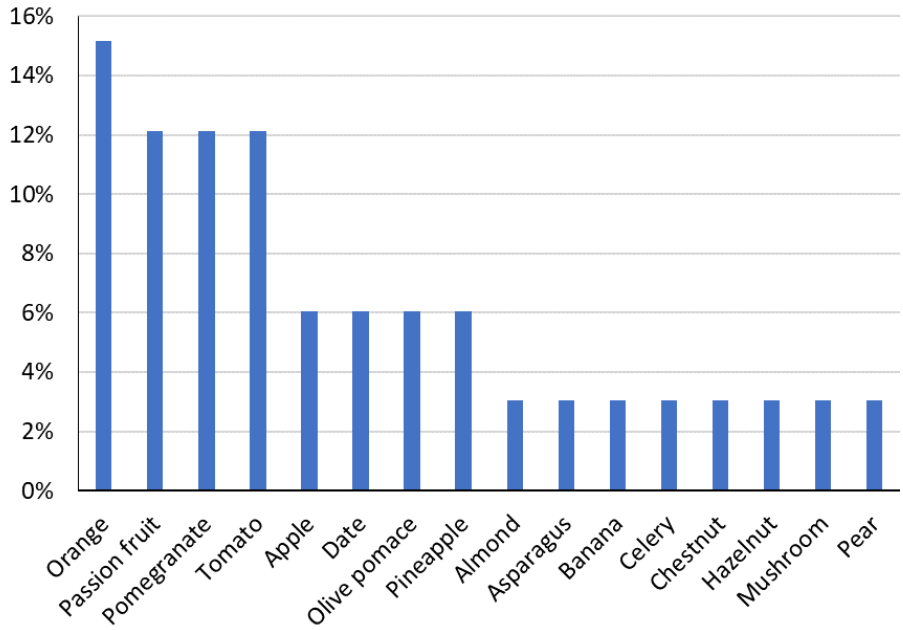


Figure 1. Percentage of research studies that used byproducts from various sources among the fruit and vegetable commodity groups in dairy food manufacturing from 2000 until July 2018 (*n* = 50 studies reviewed).

Byproducts from meat, fish and seafood contain high amounts of protein, which may be less interesting in dairy food manufacturing as they already contain this compound in their matrix. However, when protein has been needed, it has mostly been obtained from

cheese whey, which is a saccharide and protein rich waste generated during cheese production [10]. Using a byproduct from the same industry as a food ingredient not only enhances the sustainability within the dairy industry, but also may translate into fewer sensory difficulties when developing the product due to the similarities of the food matrices.

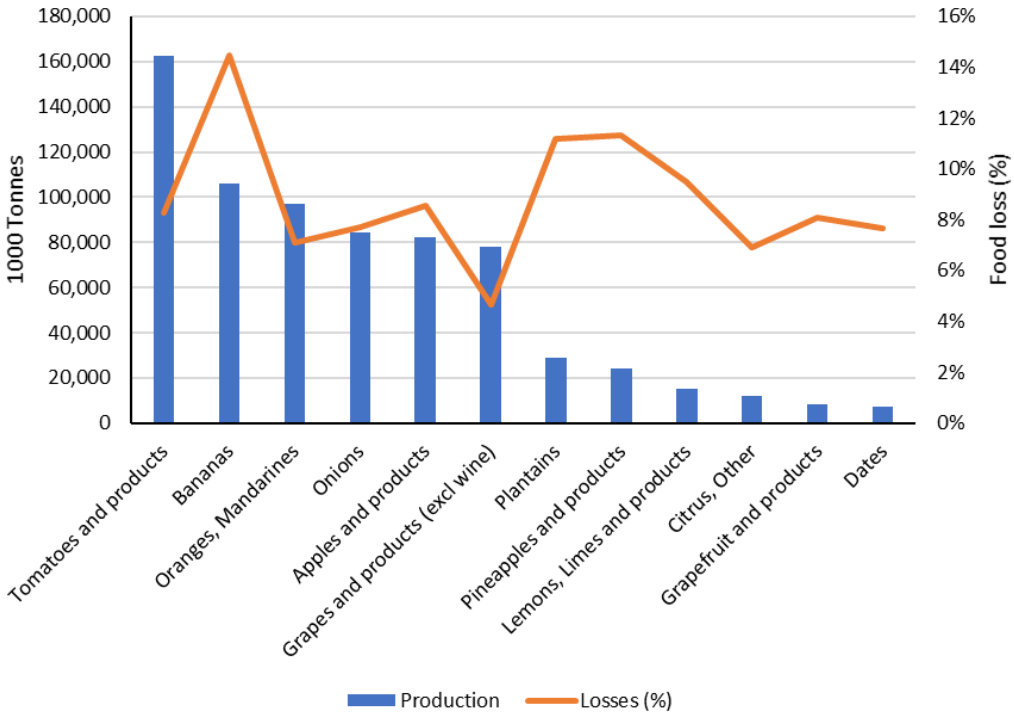


Figure 2. Worldwide food production (1000 tonnes) and its corresponding food loss (%) generated during storage and transportation within the fruit and vegetable commodity groups in 2013. Data obtained from the latest Food Balance Sheets, accessed in 2018 [9].

4. Approaches in the Application of Food Byproducts in the Dairy Industry

The exploitation of byproducts generated during food processing or discarded produce as a source of functional compounds and their application in other foods is very much desirable as part of a waste management system [11]. In this review, we divide the applications of food byproducts in dairy foods in two categories: those with technical purposes, which include the improvement of shelf life, safety, stability, sensory quality, etc.; and those with biological purposes, which aim to enhance health-promoting effects for their conversion into functional foods. A summary of the applications so far proposed is shown in Table 1.

Table 1. Dairy foods found in the literature (from 2000 to July 2018, *n* = 49) developed using food processing byproducts as sustainable ingredients.

Dairy Product	Food Industry	Byproduct	Doses	Function	Reference
Dairy beverage	Vegetable	Mushroom residue	1, 2 and 3 g/kg	Technological (antioxidant) Health-promoting (source of phenols)	Vital et al., 2017 [12]
		Olive vegetable water	100 mg/L to 200 mg/L	Health-promoting (source of phenols, probiotic protection)	Servili et al., 2011 [13]
Fermented milk	Cereal	Rice bran	1% to 3%	Health-promoting (source of fiber and phenols, probiotic protection)	Demirci et al., 2017 [14]
	Dairy	Whey protein	2%	Technological (texturizing agent) Health-promoting (source of protein)	Akalin et al., 2012 [15]
		Whey protein and buttermilk	0% to 100% replacement of skim milk powder	Technological (texturizing agent) Health-promoting (source of protein)	Saffon et al., 2013 [16]
		Whey protein	8% to 14%	Technological (texturizing agent) Health-promoting (source of protein, probiotic protection)	Zhang et al., 2015 [17]
	Fruit	Chestnut flour	2%	Health-promoting (source of phenols, probiotic protection)	Ozcan et al., 2016 [18]
		Apple	1%	Health-promoting (source of fiber, probiotic protection)	Do Espírito Santo et al., 2012 [19]
		Apple pomace	2.5% to 10%	Health-promoting (source of fiber)	Issar et al., 2016 [20]
		Banana	1%	Health-promoting (source of fiber, probiotic protection)	Do Espírito Santo et al., 2012 [19]

Dairy Product	Food Industry	Byproduct	Doses	Function	Reference
		Pineapple peel powder	1%	Technological (texturizing agent)	Sah et al., 2016 [21]
		Pineapple peel powder	1%	Health-promoting (probiotic protection)	Sah et al., 2015 [22]
		Passion fruit peels	1%	Technological (texturizing agent)	Espírito-Santo et al., 2012 [23]
		Passion fruit peels	0.7%	Health-promoting (source of fiber)	Do Espírito Santo et al., 2012 [24]
	Vegetable	Passion fruit	1%	Health-promoting (source of fiber, probiotic protection)	Do Espírito Santo et al., 2012 [19]
		Passion fruit peels	1%	Health-promoting (source of fiber)	Perina et al., 2015 [25]
		Okara	3% to 10%	Health-promoting (source of fiber)	Chen et al., 2010 [26]
		Olive pomace	100 mg/L TPC	Health-promoting (source of phenols, probiotic protection)	Aliakbarian et al., 2015 [27]
	Winery	Wine pomace extract	100 mg/L TPC	Health-promoting (source of phenols, probiotic protection)	Aliakbarian et al., 2015 [27]
		Grape marc flour	10, 20 and 50 g/L	Health-promoting (source of phenols, probiotic protection)	Aliakbarian et al., 2013 [28]
		Wine pomace extract and flour	1% to 3% 1% to 2%	Technological (antioxidant, colorant) Health-promoting (source of fiber and phenols)	Tseng and Zhao 2013 [29]
		Wine pomace extract	788 mg GAE/100 g	Health-promoting (source of phenols, probiotic protection)	Dos Santos et al., 2017 [30]
		Wine pomace flour	10, 20 and 50 g/L	Health-promoting (source of phenols, probiotic protection)	Frumento et al., 2013 [28]

Dairy Product	Food Industry	Byproduct	Doses	Function	Reference
Yogurt	Cereal	Wheat bran	1.5%	Health-promoting (source of fiber)	Hashim et al., 2009 [31]
		Rice bran	0.2% to 0.6%	Technological (colorant)	Nontasan et al. 2012 [32]
	Dairy	Whey protein	3.3, 5 and 10 g/L	Technological (texturizing agent) Health-promoting (source of protein)	Sandoval-Castilla et al., 2004 [33]
	Fruit	Date byproducts	1.5% to 4.5%	Health-promoting (source of fiber)	Hashim et al., 2009 [31]
		Orange peels, pulp, seed powders	1% to 3%	Technological (texturizing agent)	Yi et al., 2014 [34]
		Orange byproducts	0.2 to 1 g/mL	Technological (texturizing agent)	Sendra et al., 2010 [35]
		Orange albedo, flavedo and pulp powders	0.6% to 1%	Health-promoting (source of fiber)	García-Pérez et al., 2005 [36]
		Pomegranate peel extract	5% to 35%	Health-promoting (source of phenols)	El Said et al., 2014 [37]
		Hazelnut skin powder	3% to 6%	Health-promoting (source of fiber)	Bertolino et al., 2015 [38]
		Pomegranate seed	25 mg/L	Technological (antioxidant)	Ersöz et al., 2011 [39]
	Marine	Fish oil	15 mL/100 g	Health-promoting (source of omega-3)	Ghorbanzade et al., 2017 [40]
		Fish oil	13 g/100 g	Health-promoting (source of omega-3)	Zhong et al., 2018 [41]
	Vegetable	Asparagus byproducts	1%	Health-promoting (source of fiber)	Sanz et al., 2008 [42]
	Winery	Grape seed extract	100 mg/150 g	Health-promoting (source of phenols)	Chouchouli et al., 2013 [43]
		Grape skin flour	0.167 to 1 g/100 g	Health-promoting (source of phenols)	Karnopp et al., 2017 [44]

Dairy Product	Food Industry	Byproduct	Doses	Function	Reference
		Grape skin flour	60 g/kg	Health-promoting (source of phenols)	Marchiani et al., 2016 [45]
		Grape seed	25 mg/L	Technological (antioxidant)	Ersöz et al., 2011 [39]
Dairy dessert	Fruit	Date byproduct	0.5, 1 and 2 ratio dried date powder/date syrup	Technological (texturizing agent) Health-promoting (source of phenols)	Jridi et al., 2015 [46]
	Vegetable	Okara	3% to 10%	Health-promoting (source of fiber)	Chen et al., 2010 [26]
Ice-cream	Fruit	Orange peels, pulp, seed powders	1% to 1.5%	Technological (texturizing agent)	Crizel et al., 2014 [47]
		Pomegranate peels	0.1% and 0.4%	Health-promoting (source of phenols)	Çam et al., 2013 [48]
	Vegetable	Lycopene from tomato byproducts	70 mg/kg	Technological (antioxidant, colorant, antimicrobial)	Kaur et al., 2011 [11]
		Carotenoids from tomato peels	1% to 5%	Technological (antioxidant, colorant)	Rizk et al., 2014 [49]
	Winery	Grape wine lees	50, 100 and 150 g/kg	Health-promoting (source of phenols)	Hwang et al., 2009 [50]
Butter	Fruit	Almond peel extract	100 ppm to 400 ppm	Technological (antioxidant)	Nadeem et al., 2014 [51]
	Vegetable	Lycopene from tomato byproducts	20 mg/kg	Technological (antioxidant, colorant, antimicrobial)	Kaur et al., 2011 [11]
		Tomato processing byproduct	400 and 800 mg/kg	Technological (antioxidant)	Abid et al., 2017 [52]
Cheese	Cereal	Corn bran	5%	Health-promoting (source of phenols)	Lucera et al., 2018 [53]

Dairy Product	Food Industry	Byproduct	Doses	Function	Reference
		Wheat bran	10 g/500g	Health-promoting (probiotic protection)	Terpou et al., 2018 [54]
	Dairy	Fluid whey	Water substitution	Technological (texturizing agent)	Erbay et al., 2015 [55]
	Fruit	Pomegranate peel	100 mL/25 g	Technological (antioxidant, antimicrobial)	Shan et al., 2011 [56]
		Orange byproduct fibers	3% to 5%	Technological (texturizing agent)	Saraç and Dogan 2016 [57]
		Pear stones	3% to 5%	Technological (texturizing agent)	Saraç and Dogan 2016 [57]
	Vegetable	Spinach	3% to 5%	Technological (texturizing agent)	Saraç and Dogan 2016 [57]
		Celery byproduct fibers	3% to 5%	Technological (texturizing agent)	Saraç and Dogan 2016 [57]
		Okara	1% to 4%	Health-promoting (source of fiber)	Chen et al., 2010 [26]
		Tomato peels	5%	Health-promoting (source of phenols)	Lucera et al., 2018 [53]
		Broccoli stems and leaves	5%	Health-promoting (source of phenols)	Lucera et al., 2018 [53]
		Artichoke external leaves	5%	Health-promoting (source of phenols)	Lucera et al., 2018 [53]
	Winery	Grape seed	100 mL/25 g	Technological (antioxidant, antimicrobial)	Shan et al., 2011 [56]
		Wine pomace, skin and seed extracts	0.1, 0.2 and 0.3 wt/vol	Health-promoting (source of phenols)	Da Silva et al., 2015 [58]
		Wine pomace flour	0.8 and 1.6 w/w	Health-promoting (source of phenols)	Marchiani et al., 2015 [59]
		Grape pomace	5%	Health-promoting (source of phenols)	Lucera et al., 2018 [53]

4.1. Technological Applications of Food Byproducts in Dairy Formulations

One of the major emerging technologies is the application of food byproducts as natural additives. The implementation of this approach could serve a double purpose. As a waste reduction measure, it would enhance sustainability and increase industrial profitability. In addition, it would be possible to fulfill the requirements of consumers concerned about chemical residues in their foods that look for clean-label and naturally-preserved healthy foods [60].

The addition of food additives is regulated under Codex Alimentarius guidelines. Therefore, food byproducts used as natural additives must consider current regulations and undertake proper authorization if necessary. In this section, we summarize the ongoing research carried out to apply food byproducts as additives in dairy products.

4.1.1. Use of Byproducts as Antioxidants

The Codex General Standard for Food Additives defines antioxidants as food additives which prolong the shelf-life of foods by protecting against deterioration caused by oxidation [61]. In dairy products, lipid oxidation produces fatty acid hydroperoxides, an intermediary tasteless and odorless compound which can further react with fatty acids leading to the formation of secondary lipid oxidation products and protein damage [62]. These reactions result in the production of off-flavors in milk and dairy products, which are described as cardboardy and metallic [63]. These off-flavors can be detected in raw or pasteurized milk, in any dairy product that has not been flavored, and especially in high-fat products such as butter or ice-cream. Therefore, changes in the properties and palatability of these products can lead to a decrease of consumer acceptability and confidence in dairy products [12].

The susceptibility of milk lipids to oxidation depends on several factors: intrinsic factors, extrinsic factors and their interrelation [62]. Intrinsic factors include the composition of the milk system, which is constituted by a complex mixture of pro-oxidants (transition metals) and antioxidants (tocopherols, uric acid, ascorbic acid), whose relative concentration in milk are related to seasonal, physiological and nutritional effects on the cow [64]. Extrinsic factors that affect lipid oxidation refer to environmental and physical factors (light exposure, temperature, pH, water activity, etc.), and to changes that occur during processing and storage (homogenization, heat treatment, fermentation, proteolysis) [65].

This way, the addition of antioxidants in milk is one of the main methods used for preventing and retarding lipid oxidation. The most commonly applied antioxidants in

dairy foods, when their use is not explicitly excluded by legislation, are ascorbates and tocopherols [66].

As an alternative to conventional antioxidants, different bioactive compounds recovered from food byproducts have been used to prevent lipid oxidation of dairy foods and increase their shelf life. These efforts have been made especially in high-fat content dairy foods, such as cheese and butter, but also in yogurts and other dairy products such as milk drinks fortified in omega-3 fatty acids, which have a higher risk of lipid deterioration. Agaricus blazei mushroom residue has been added to milk fortified in omega-3 fatty acids, which decreased lipid oxidation when subjected to photooxidation [12]. Wine grape pomace also proved to delay lipid oxidation in yogurt [29], whereas grape seed and pomegranate peel extracts have been applied effectively to protect against lipid oxidation in cheese during storage [56].

Butter contains the largest amount of fat among dairy foods (approximately 80%) and can be kept well for at least 20 days if correctly stored at 10 °C, protecting it from moisture evaporation and light induced photooxidation. However, during cold storage, autoxidation is the main cause of deterioration, which depends on the copper present in the product [67]. Antioxidants from tomato processing byproducts were used as agents against lipid peroxidation in conventional and traditional Tunisian butter, showing a protective action during 4 months of cold storage [11,52]. A protective effect against lipid oxidation during 3 months of cold storage was also shown by adding almond peel extract in whey butter, which contains a higher concentration of unsaturated fatty acids that are more vulnerable to oxidative breakdown [51]. The addition of almond peel extract allowed whey butter storage up to 3 months which showed no significant differences in acceptability scores against milk butter [51].

It is relevant to consider the dosage of the extract added to the food product, as it can affect both the antioxidant behavior of the extract and the final sensory acceptability of the food. The effectiveness of tomato processing byproducts as antioxidants in butter was found to be dose dependent: lower amounts of the extract (400 mg tomato processing byproduct/kg butter) considerably inhibited the formation of oxidation products, extending the shelf life of the product up to two months; whereas greater concentrations of tomato processing byproducts (800 mg tomato processing byproduct/kg butter) showed pro-oxidant properties with detrimental effects on the stability of the butter [52]. This change in the antioxidant/pro-oxidant capacity of certain compounds was also described in other studies [68], which observed that extracts with

high concentrations of b-carotene lost their antioxidant effect becoming pro-oxidant, possibly due to long-chain-oxidized products of the carotenoid.

The addition of byproduct extracts can lead to either positive or negative effects on the sensory properties of the final product, depending on the dosage, the type of recovered byproduct compounds and the food matrix in which it is incorporated. In ice-cream, the addition of tomato peel carotenoid concentrations of 4% or higher lowered acceptance scores for flavor, texture, melting quality and color [49]. Tomato byproducts used in a different matrix, butter, significantly improved the product's appearance after 4 months of cold storage compared to the control butter [11]. Different byproduct extracts, such as grape and pomegranate seed extracts, decreased fat deterioration in sheep yogurt, but their sensory profile was significantly less acceptable than the control samples immediately after yogurt manufacture and after 14 days of storage [39].

4.1.2. Use of Byproducts as Antimicrobials

Preservatives are food additives that prolong food shelf life by protecting against deterioration caused by microorganisms. Different types of preservatives include: antimicrobial antimould antirope and antimycotic agents, antimicrobial synergists, bacteriophage control agents and fungistatic agents [61]. Food byproducts have been used as preservative agents with antimicrobial activity to ensure that manufactured dairy foods remain unspoiled and safe during their whole shelf-life.

Several studies have shown that food byproducts can be used against spoilage and pathogenic bacteria without interfering with the viability of starter cultures and other microorganisms involved in fermentation processes, ensuring that the quality of the developed products is maintained. The bacterial concentrations required in yogurts and fermented milks by the Codex Alimentarius (107 CFU/g) were still met when different byproducts were added into the food matrix (grape pomace flour and extracts, grape skin and seeds, hazelnut skins, pineapple peels, pomegranate seeds, passion peels, etc.) [22,24,27,28,38,39,45]. In cheese, there is less information on the effect of byproduct addition on molds, yeasts and bacteria during ripening, even though they are essential for the correct development of cheese flavor and texture [69,70]. Winemaking byproducts have been added in Toma-like and cheddar cheese products, showing that their addition did not interfere with starter and nonstarter bacteria nor with cheese proteolysis [59]. Therefore, there is an opportunity to study whether the addition of recovered compounds interferes during ripening in other cheese types that involve the growth of different molds in the cheese rind (soft cheese, natural rind cheese, etc.).

4.1.3. Action Against Dairy Food Spoilage Microorganisms

Milk spoilage is primarily due to the growth of psychrophilic microorganisms that trigger lipolysis and proteolysis reactions of milk fatty acids and proteins, respectively [71]. Lipolysis of milk lipids to free fatty acids and partial glycerides contributes to the desirable flavor of milk and other dairy products, but when present in high concentrations, it can lead to the development of off-flavors. These are described as rancid, butyric, bitter, unclean, soapy and astringent [72]. Once lipolysis produces detectable off-flavors it is not possible to remove them from the product [73]. In addition, the hydrolysis of milk proteins produced by proteases from Pseudomonads, Aeromonads, Serratia and Bacillus spp. also result in the release of off-flavors due to the production of bitter peptides and milk gelation and coagulation [74–76].

Milk spoilage is mediated by lipases that are naturally present in milk (lipoprotein lipase) or by lipases and proteases from psychrophilic bacterial contamination occurring during milking, storage and transportation that result in the destabilization of milk during cold storage [62,77]. One of the most important properties of these bacterial enzymes is their heat stability. This is because most of them can retain at least some of their activity after pasteurization or ultra-high temperature (UHT) treatment, even though bacteria are destroyed [63,78]. Therefore, it is important to develop good practices and strategies to minimize the risk, such as achieving a low microbial count in milk before pasteurization as the action of the residual enzymes during storage will shorten the milk's shelf life [74].

Quality issues and defects associated with excessive lipolysis in dairy products include rancid flavors and poor foaming capacity in pasteurized milk, rancid flavor due to increasing free fatty acids in UHT milk, and spoilage of milk powder during storage. Flavor defects in cheese and butter can be caused by lipolysis before or after manufacture, whereas yogurt is less susceptible to lipolysis defects due to a combination of factors such as low pH, low storage temperature and short shelf life [73].

Although different applications of recovered food byproducts are being studied to valorize them as novel food ingredients, there is a lack of information on the effect of the addition of these extracts in the lipolysis or proteolysis of dairy foods. This should be considered, as some additives, such as pepper, promote lipase activity in cheese, producing soapy and rancid off-flavors [73]. To our knowledge, there is only one study that described the effect of byproducts on the hydrolysis of lipids in dairy foods. Tomato processing byproducts were used in butter and ice-cream to prevent lipolysis during 4 months in refrigerated storage [11]. A significant decrease in the liberation of free fatty

acids was observed in lycopene added butter after 3 months compared to control butter, suggesting that this extract may exert a protective action against lipolysis.

4.1.4. Action Against Foodborne Pathogens in Dairy Foods

The milk matrix is an ideal media for microorganism proliferation. This also includes pathogenic bacteria, where mycobacteria, Brucella sp., Listeria monocytogenes, Staphylococcus aureus and enterobacteria (including toxigenic Escherichia coli and Salmonella) are the most frequently found pathogenic bacteria in dairy foods [76]. The origin of pathogen proliferation can be either endogenous (from udder infection) or exogenous (contact with contaminated environment) [79]. Therefore, implementation of Hazard Analysis and Critical Control Points (HACCP) and quality assurance programs through European Union (EU) directives (2004/41/EC, EU 605/2010) on milk hygiene and public health conditions have been put into practice to ensure food safety [80].

Milk heat treatment, such as pasteurization or UHT processes, kill pathogenic bacteria. However, inadequate pasteurization or post-pasteurization contamination can cause milk re-contamination if sanitation measures in the processing plant are not sufficient, leading to food poisoning incidences [74]. Outbreaks of food-borne illnesses have been mainly linked to the consumption of raw milk or products made of unpasteurized milk such as raw milk cheeses, whose consumption is continuously growing [81]. Besides not using heat treatment, traditional raw milk cheese producers may not use starter cultures in their elaboration process, which increases the risk of pathogen multiplication as the competitive activity of the lactic acid starter is eliminated [82]. In this sense, the addition of preservatives to dairy products is principally used in cheese. Preservatives may be added during cheese production and ripening to all the edible part of the cheese or only for rind treatment [66,83].

The number of dairy food infection outbreaks due to pathogen contamination of other dairy foods is less common, although some cases have been reported for yogurt and fermented milks [84]. In these products the acidity of the matrix acts as a barrier to bacterial growth. However, milk must be pasteurized as some pathogens, such as E. coli 0157:H7, can be tolerant to the acid environment [85].

Many studies have analyzed the antimicrobial and antimycotic in vitro properties of extracts recovered from food byproducts. The antimicrobial action against foodborne pathogens has been associated with the polyphenols of plant based byproducts, which may penetrate the cell wall causing membrane disruption, damage of membrane proteins and enzymes, and structural changes that lead to bacterial death [86–88].

The number of studies analyzing the efficacy of byproduct polyphenolic extracts included in the dairy food matrix on food pathogen control is still limited. Pomegranate peel and grape seed extracts proved to be effective natural preservatives against Listeria monocytogenes, Staphylococcus aureus and Salmonella enterica in cheese [56]. Pathogen counts in cheese significantly decreased with the byproduct extract treatments. However, the cheese matrix required higher concentrations of the byproduct extracts to efficiently deliver the antibacterial effect compared to the in vitro analyses performed in the culture medium. This could be explained by the effect of the micro-architecture of the food matrix. Microbial growth occurs in the aqueous phase of food and is affected by food structure which can restrict the mobility of bacteria. In cheese, which is a gelled emulsion, fat and protein content together with low water content may act as a protective barrier between the bacteria and the extracts, requiring higher concentrations of preservatives to control the growth of pathogens [89].

The addition of herbs and spices in cheese has been part of the cheese culture in many countries for centuries. Some examples include the French Banon covered in chestnut leaves, or the Spanish Majorero cheese with sweet pepper. In this sense, the antimicrobial effect of herbs and spices and their application as cheese preservatives has been more commonly studied [90,91]. This tradition could be used as a cultural advantage for the application of plant-based byproducts as preservative and flavoring agents in innovative cheese developments. As consumers already feel familiar with this type of cheese products, it could increase product acceptability and facilitate its introduction into the market.

4.1.5. Use of Byproducts as Colorants

Colorants are food additives that add or restore color in foods [61]. Their role is involved in the improvement of the appearance and color of foods, and in the maintenance of their natural color during processing and storage [92]. Color stands as one of the most important quality attributes for the food industry, as it directly affects consumers' acceptance and food selection [93].

Current market trends include the substitution of synthetic colorants for natural compounds, which has been motivated by consumers' concern about the safety of synthetic food dyes (side effects, toxicity and allergic reactions), and by the possible health-promoting benefits of natural pigments [94].

Fruit and vegetable byproducts have become an important source of natural pigments as they are colored by green chlorophylls, yellow-orange-red carotenoids, red-blue-purple anthocyanins and red betanins [95].

Anthocyanins have been widely extracted from various plant-based foods and byproducts, such as radishes, red potatoes, red cabbage, black carrots, purple sweet potatoes, coffee husks, berries, winery byproducts, etc. [96–98]. However, their use as food colorants has been limited. The list of anthocyanin colorants in the Codex Alimentarius includes only grape skin extract (E163), and in the FDA, "grape color extract" and "grape skin extract" (enocyanin) [61,99].

Anthocyanin application in dairy foods comes with a range of unique coloring challenges, as their stability is affected by changes in pH, fat content in the dairy matrix and manufacturing and storage conditions including extreme temperature and light exposure [97]. Moreover, their use may add specific flavors associated with phenolic compounds. This is the case in some studies where the addition of wine byproducts in yogurt and fermented milks for polyphenol enrichment and color improvement resulted in a decrease in overall liking due to a predominant astringent sensation [29,44,45]. This problem is solved by adding sucrose or other ingredients to the basal recipe to eliminate the astringency. Higher sensory scores in flavor and overall acceptability were reported in wine pomace-fortified fermented milks compared to control samples [30]. The greater acceptability of the polyphenol-fortified samples was probably due to the influence of the intensified color on the perception of taste. Other satisfactory applications of food byproducts as colorants have been reported using anthocyanins from grapes and beetroot betalains. The coloring compounds proved to be stable in semisolid petit-suisse-like cheese probably due to its low water content, slightly acid pH and the low temperature and light-impermeable packaging during storage [100].

Carotenoids stand as the major group of compounds used as coloring agents. Their use is widely extended, and the number of authorized carotenoids used as colorants varies depending on each country. Most commercial carotenoids are produced synthetically (β-carotene, astaxanthin, canthaxanthin and zeaxanthin), although some are obtained from natural sources (annatto, paprika, saffron, marigold, tomato, algae) and microbial fermentation [95]. Extraction of lycopene from tomato processing byproducts has been optimized and registered as the food color "E160d" in Europe [61]. In dairy foods, lycopene from tomato byproducts has been applied in the coloring of butter and ice-cream showing a stable reddish color for up to 4 months [11,49].

4.1.6. Use of Byproducts as Texturizing Agents

Texturizing agents are used to add or modify the overall texture and mouth feel of food products by providing creaminess, thickness, viscosity or a stable structure. This category comprises a wide range of food additives including emulsifiers, stabilizers, thickeners and bulking agents [61]. Texturizing agents are commonly used in dairy products. Hydrocolloids are used for stabilizing and thickening purposes in fermented milks, milk drinks, dairy desserts, cream and ice-cream. Phosphates and coagulation agents are also permitted as stabilizers and to aid in the curdling of milk in cheese production, respectively [66,101].

Most hydrocolloids used in dairy foods come from natural origin as they are manufactured by isolation from seaweeds and plant cells [102]. Moreover, many of these hydrocolloids are extracted from plant food wastes, such as pectin, which is commonly isolated from apple pomace and citrus peels, as well as from other fruit and vegetable byproducts such as passion fruit peels, rapeseed cake, olive pomace, grape pomace, onion hulls, etc. [103–106]. Their application in dairy foods as isolated ingredients is increasing which is a step forward in valorizing underused fractions. However, the isolation of specific compounds generates once again other byproducts. To improve economic and environmental sustainability within the food chain, newer approaches trying to use byproducts as whole ingredients without further processing should be developed. This represents a harder challenge as byproducts used as ingredients comprise a much more complicated matrix than an isolated compound, which could lead to problems associated with product stability and unwanted interaction with other compounds.

In this sense, fewer studies have reported the use of food byproducts as whole ingredients with texturizing purposes. Some examples include the use of liquid fluid whey instead of the generally used powdered form, which showed promising results on the physical quality of white cheese powder [55]. Dietary fiber from orange byproducts was used to maintain the texture of lemon ice-cream when reducing its fat content by 50% [47], and as fat replacers in low-fat yogurt [34]. The authors showed that reducing particle size of the orange dietary fibers by micronization increased their water and oil holding capacities, which are also important functional properties in relation to the facilitation of digestion and absorption of nutrients in the body.

Texture, rheological parameters and the microstructure of yogurt gels have been analyzed when adding different fibers. A gel structure with large pores and reduced cross-linking between casein micelles in yogurts was observed with 1% of pineapple

peel powders, which was associated with lower yogurt firmness and weak rheological properties due to the incompatibility between milk proteins and polysaccharides from the pineapple peel powders [21]. Although the presence of fiber particles always alters yogurt structure, high amounts of passion fruit peel powders or orange byproduct fibers counterbalanced this negative effect and strengthened the casein network possibly due to the water absorption capacity of the fibers [23,35]. This effect of fiber dose and fiber type was also observed in the firmness and spreadability parameters of butter fortified with fibers (from 3% to 5%) from vegetal and fruit wastes: stone pear, celery roots and leaves, spinach and orange albedo [57].

4.2. Health-Promoting Applications of Food Byproducts in Dairy Formulations

Advances in nutrition and medical science have shown that both nutrients and non-nutrient components of foods are important for maintaining good health. This, together with the increasing knowledge of the biochemical structure and functions of bioactive compounds and their effects on the human body, have led to the rise in popularity of functional foods [1]. Although there is no universally accepted definition of functional foods, they can be described as foods that claim to have health benefits beyond basic nutrition [107]. Functional foods are an increasing market segment aimed at consumers who are taking greater responsibility for their own health and well-being [108]. Simultaneously, diet-related illnesses, such as cardiometabolic diseases including coronary heart disease, stroke, type 2 diabetes and obesity stand as one of the greatest global health and economic burdens of our times, accounting for 31% of all deaths worldwide [109,110]. As part of a healthy dietary pattern and lifestyle, functional foods stand as a promising strategy in non-communicable disease prevention.

Within a scope of food waste reduction, much progress has been made using food byproducts as sources of bioactive compounds or as functional ingredients by themselves for the development of dairy functional products. It must be noted that new food ingredients developed from food byproducts that have not been used for human consumption within the EU prior to 1997 must be subjected to official review and approval according to the European Regulation on Novel Foods and Novel Food Ingredients (258/97). This section summarizes the research that has been carried out using byproducts in the manufacture of health-promoting dairy foods.

4.2.1. Use of Byproducts in the Development of Functional Dairy Foods Containing Polyphenols

Polyphenols are secondary metabolites that are synthesized during normal plant development and in response to stress conditions [111]. Plant phenolics include phenolic acid and its derivatives, flavonoids, lignans and stilbenes [112]. Although phenolic compounds are not considered nutrients, several biological and pharmacological activities have been attributed to dietary polyphenols, including antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-microbial and anti-carcinogenic effects [113]. These properties play a relevant role in the prevention of several major chronic diseases associated with oxidative stress, such as cardiovascular diseases, cancers, type II diabetes, neurodegenerative diseases and osteoporosis [114]. In this sense, the health-protecting capacity of plant phenolics has become of great interest for researchers, the food industry and consumers.

Peels, husks, hulls, pods and bran are major processing byproducts of the fruit, vegetable and cereal industry that are considered sources of polyphenols. They have mostly been applied for polyphenol fortification in yogurt and fermented milks. Namely, winemaking byproducts have been used as the main source of polyphenols, including different flours and extracts from grape pomace and other selective fractions, such as grape skins and seeds. This could be justified both by the fact that black grapes stand among the richest dietary sources of polyphenols [115,116] and by the high amount of grape losses generated during processing and conversion into wine, storage and transportation, which reached 3.6 million in 2013 [9]. Other byproducts from fruits, nuts, vegetables and cereals have also been used as sources of polyphenols for the development of fermented milks and yogurts. These byproducts included pomegranate seeds and peels, almond peels, hazelnut skins, olive pomace and rice bran [12,14,18,37–39].

The addition of polyphenols to dairy foods other than yogurt and fermented milks has received less attention. Wine pomace byproducts have also been the major source of polyphenols used to formulate cheese [53,56,58,59] and ice-cream [50], although other phenol byproduct sources have recently been studied in spreadable cheese (tomato peels, broccoli stems and leaves, corn bran and artichoke external leaves) [53]. The application of broccoli stems in spreadable cheese is particularly interesting, as it could increase glucosinolate content in the product, which are compounds also associated with beneficial health properties [117].

Wine pomace flours have been directly used as ingredients in fermented milk and yogurt development [28,29,45]. The advantage of using powders instead of extracts from the

byproducts is that less processing is required, which is a more sustainable approach as it consumes less energy and does not generate secondary byproducts. On the other hand, the disadvantage of using powders is that higher doses are needed to achieve significant polyphenol fortification levels, which penalizes the organoleptic properties of the products. That is why many studies have switched towards using extracts from wine pomace [27,30,39,43].

In this sense, product formulations with a compromise between functional properties and sensory acceptance need to be developed. In foods, polyphenols may contribute to the bitterness, astringency, color, flavor and odor of the products [118]. Polyphenols are associated with the precipitation of salivary glycoproteins and mucopolysaccharides on the tongue, resulting in roughness and dryness on the palate [119]. This is why several studies have reported an inverse relation between polyphenol dosage and consumer acceptance in dairy products [29,38,44,59]. A decrease in the overall acceptance of yogurts with 6% added polyphenols from grape skin flours [45] and of yogurts with 1% and 2% grape pomace powders [120] was observed compared to yogurt formulations with lower doses due to flavor, texture and consistency parameters.

In order to mask the negative sensory effects of polyphenols, several researchers have evaluated their use together with other ingredients. In yogurt and fermented milk fortification with wine pomace byproducts, the best acceptance scores were obtained when polyphenols were added in combination with sucrose (5%), oligofructose (0.5% to 0.667%) or grape juice (0.167% to 0.5% and 15%) [30,44].

Besides the sensory and quality challenges associated with the addition of polyphenols in the dairy matrix, the bioaccessibility and bioavailability of the bioactive compounds should be taken into consideration to truly establish whether the wanted biological health effects are being met. Evidence suggests that polyphenols are absorbed in a relatively low amount. Most polyphenols are poorly absorbed in the gastrointestinal track, reaching the colon where they are metabolized by colonic microbiota. These metabolites are responsible for the biological activities associated with polyphenols [121]. The resulting bioactivity will depend both on the interactions between polyphenols and other macromolecules (lipids, proteins and carbohydrates), which will affect their bioaccessibility and bioavailability, and on the specific microbiota present in each individual's colon, which can give rise to different phenolic metabolites [117,122]. Therefore, further knowledge on the food matrix and food interaction together with the role of gut microbiota on the metabolism and activation of the dietary constituents, will provide original ideas for the development of new functional foods, in which a

combination of plant-derived food ingredients with the appropriate bacterial strains will lead to improved biological activity for a specific food product [117].

4.2.2. Use of Byproducts in the Development of Functional Dairy Foods Containing Dietary Fiber

Plant-derived byproducts, such as seed, skins, pods, peels, pomace, hulls, husks, cores, stores, etc., are known sources of bioactive compounds and nutrients including dietary fiber [1,123], whose caloric value has been estimated at 2 kcal per g (FDA, 2018). The European Food Safety Authority (2010) [124] defines this nutrient as non-digestible carbohydrates, including non-starch polysaccharides, resistant starch and oligosaccharides, and lignin. A terminology often encountered is the classification of dietary fiber as “soluble” or “insoluble” [125]. Therefore, the physicochemical properties of the different dietary fibers can be determinant when selecting their applications.

Worldwide and country-specific governmental institutions confirmed that there is evidence of health benefits associated with consumption of diets rich in fiber-containing foods, and recommendations on the intake of dietary fiber range between 25 g to 38 g per day [126]. Health benefits have been related to a reduced risk of coronary heart disease, intestinal disorders, type 2 diabetes and improved weight maintenance [127–129].

Product innovations have been focused on increasing the fiber content of dairy foods with two purposes: to help consumers achieve the daily recommended intake of dietary fiber, and as a marketing strategy to add a nutritional claim on the food package. The European Parliament and Council, (2006) (Regulation No. 1924/2006) [130] stated that the nutritional claim “source of fiber” or “high in fiber” can only be made when the product contains at least 3% (or 1.5 g of fiber per 100 kcal) or 6% (or 3 g of fiber per 100 kcal) dietary fiber, respectively. Bearing this in mind, several researchers have used dietary fiber concentrations ranging from 2.5% to 10% to evaluate its feasibility as an ingredient in dairy products, as an increase in concentrations of dietary fiber in foods can lead to changes in the resultant nutritional, textural, rheological, and sensory properties of the developed products [131].

Development of dairy foods fortified with high contents of dietary fiber have mostly been carried out in yogurt and fermented milks. Available studies have used a wide variety of plant-origin sources derived from fruit and vegetable industry byproducts. Water soluble soybean polysaccharides from okara, which is the byproduct of tofu,

soymilk and soybean protein isolate, were used in the development of ice-cream, pudding and a milk-based beverage [26]. Optimal sensory acceptance of products was achieved in milk beverages and pudding with 4% dietary fiber, and in ice-cream with 2% dietary fiber. Higher dietary fiber doses were rejected as consumers considered the foods too thick when evaluated using Just About Right (JAR) scales. In fermented milks and yogurts, fiber from apple pomace (3% to 10%), date byproducts (1.5% to 4.5%) and hazelnut skins (3% to 6%) were used in the development of dietary fiber-fortified foods [20,31,38]. In these cases, optimal sensory acceptance of the products was obtained at 3% fiber addition from hazelnut skins and dates, and 5% fiber addition from apple pomace.

These examples demonstrate that it is possible to increase the doses of dietary fiber for the development of dairy products with optimal sensory acceptance that could be labeled as “source of fiber” on their package. Achieving a “high in fiber” label may be more problematic both from a technological and biological point of view, as textures may be too thick, and consumption of high content dietary fiber products may cause potential secondary effects from carbohydrate fermentation including bloating, distension, flatulence, loose stools and increased stool frequency [132].

Other studies have successfully fortified yogurts and fermented milks with dietary fiber from other byproduct sources, such as orange, passion fruit and asparagus byproducts, but in lower doses (0.6%–1%), which also contribute to increasing the daily intake of dietary fiber in consumers’ diets and potentially promote associated health benefits, but do not achieve a nutritional claim [24,25,36,42].

Lower doses of dietary fiber from food byproducts have also been used in the development of fermented milks to protect probiotics and enhance their viability. It is well documented that probiotic bacteria grow slowly in milk because they are devoid of proteolytic enzymes [133]. Therefore, milk solids supplementation is a good practice to improve probiotic growth during fermentation and favor their viability in the product [134]. Rice bran, olive and wine pomace, cheese whey, pineapple, apple, banana and chestnut byproducts have been used for probiotic protection in fermented milks [13,14,17–19,22,27,28,30]. To our knowledge, the only attempt to use byproducts to promote probiotic viability in a different dairy matrix has been using wheat bran in cheese [54].

In addition to enhancing probiotic viability, probiotic strains can act synergistically with specific types of fiber during fermentation to improve the fatty acid composition in fermented milks. This is because some strains of bacteria are able to change the fatty

acid profile of milk during fermentation and produce functional fatty acids, including conjugated fatty acids, as the result of their growth and metabolism [135]. Moreover, the addition of other ingredients into the milk, such as prebiotics, can further increase the content of functional fatty acids in fermented milks [136]. In a study using Lactobacillus acidophilus and Bifidobacterium animals subsp. lactis strains, the addition of banana fiber significantly increased α-linoleic acid content, whereas passion fruit fiber promoted the increase of conjugated linoleic acids in probiotic yogurts [19]. Therefore, further studies should focus on the probiotic-fiber synergistic effect to improve the nutritional quality of dairy products, as the application of dietary fiber from fruit byproducts could be a more cost-effective and sustainable option than the addition of conjugated linoleic acids precursors and commercial soluble fiber that are normally used to improve the fatty acid profile of yogurts.

4.2.3. Use of Animal Origin Byproducts in the Development of Functional Dairy Foods

Ingredients derived from animal origin byproducts have been used in the development of dairy foods fortified in omega-3 fatty acids and dairy foods with a high protein content. Fish oil extracted from fish wastes is an excellent source of many unsaturated fatty acids, including long chain omega-3 cis-5,8,11,15,17-eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) [137]. However, its application in food formulations fortified in omega-3 is limited because of its easy oxidation and strong odor [41]. Successful attempts to develop yogurts containing omega-3 that had sensory attributes similar to plain yogurt were obtained by encapsulating fish oil in nano-liposomes [40], and adding a fish oil/γ-oryzanol nanoemulsion to yogurt [41].

Functional dairy foods using whey proteins obtained from cheese processing have been widely used as fat replacers and in the development of dairy foods with the nutritional claim “source of protein” or “high in protein” that are already commercially available. Problems associated with using whey proteins and sodium caseinate as fat replacers in yogurt included powdery taste, excessive acid development from lactose fermentation, higher syneresis, excessive firmness and grainy texture [33]. Improved texture parameters have been achieved in low fat yogurts and low-fat probiotic yogurts with added whey-buttermilk protein aggregates, whey protein concentrate and heat-treated whey protein concentrates [15–17].

5. Sensory Challenges and Consumer Perspective of Using Byproducts in Dairy Foods

Towards the end of the nineties, consumer acceptance was both referred to as the key success factor for functional foods and the top priority for further research [138]. Since then, several authors have tried to cover this research gap, focusing on sensory and consumer science of functional foods. The latest findings have shown that the perceived importance of food for health is still increasing, but that consumers' critical attitude towards functional foods is also increasing, which translates into lower willingness to compromise on taste for health [139].

The current approach to the development of functional foods using byproducts as novel ingredients has focused on selecting specific concentrations of the byproducts to improve the technological and health-promoting properties of the products, and afterwards, evaluating their sensory acceptance. Not all studies included sensory or consumer analyses of the developed product, and when done, many studies were short on the number of volunteers to achieve significant conclusions. This context reflects that the gap in sensory and consumer research is still present, and that further analyses in this field need to be included in the academic and industry sectors to respond to the good-tasting functional food demand.

The challenge of developing good-tasting functional foods within the dairy industry increases when using food byproducts. Several authors have reported organoleptic issues associated with the use of byproducts in dairy foods, mainly due to the acrid, astringent, bitter or salty off-flavors inherent to plant-based phytonutrients [29,45,140]. In addition, there is a lack of information on the consumer's perspective of using food byproducts as ingredients in other foods. The possible concerns regarding food quality and safety that may arise, as well as the importance of sustainability as a driver in food choice should be investigated.

Bearing in mind that the functional food segment is a highly competitive and continuously changing market, using food byproducts as ingredients could be regarded as an opportunity for product differentiation. Further research should focus on the development of innovative flavors and textures to achieve more palatable foods, as well as on suitable marketing strategies to place these healthy and sustainable products in the market.

6. Conclusions

The applications described in this review show the high potential of valorizing food byproducts for the development of innovative and healthy dairy foods. Byproducts used as sustainable ingredients or sources of bioactive compounds have been shown to be effective for a wide range of technological and nutritional purposes in dairy product manufacture. This approach not only takes a step forward to waste reduction in the food chain, but also offers new ways to diversify the production of dairy foods, creating the possibility of satisfying a market niche based on functional and sustainable products. It is crucial that food technologists, nutritionists and sensory scientists work together to face the challenge of developing more palatable and well accepted foods. Moreover, it is necessary to analyze the consumer's perception and potential food safety concerns on the use of byproducts in food formulations, and specifically, for the dairy food segment.

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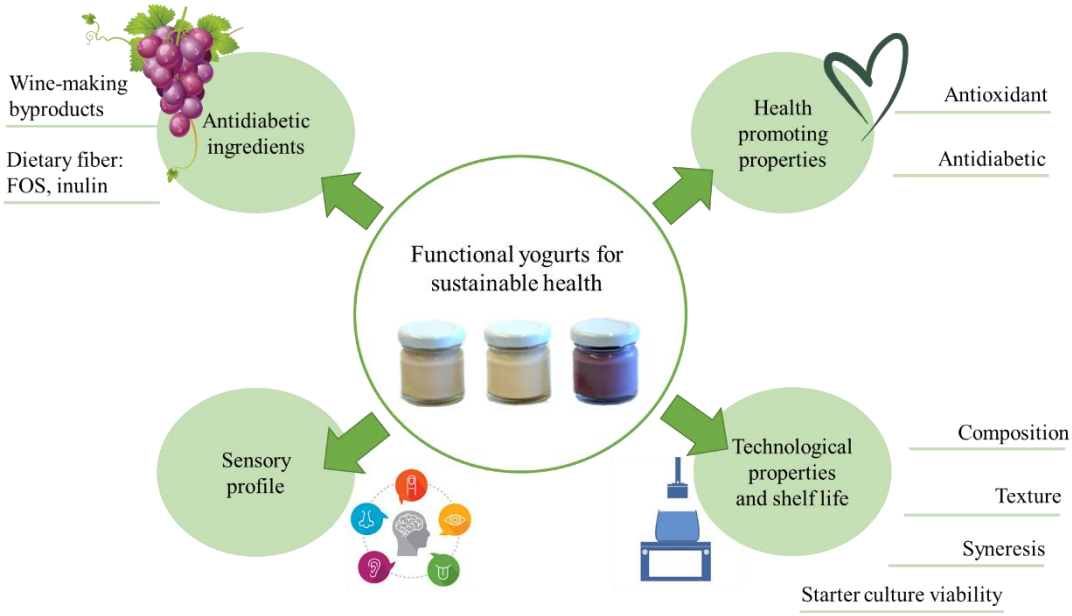
CHAPTER 2

This chapter provides a holistic analysis of the quality of yogurts containing wine-making byproducts:

- In Study 1, the technological stability, biological properties and organoleptic properties of yogurts containing grape pomace, grape seed and grape skin were analyzed.
- In Study 2, consumer's perception towards the use of wine-making byproducts as novel ingredients was evaluated.

Study 1: Wine-making byproduct extracts and dietary fiber in the development of yogurts for sustainable health

Iriondo-DeHond, M., Blázquez-Duff, J.M., del Castillo, M.D., & Miguel, E. 2019. Wine-making byproduct extracts and dietary fiber in the development of yogurts for sustainable health.



Wine-making byproduct extracts and dietary fiber in the development of yogurts for sustainable health

Maite Iriondo-DeHond [1,2], José Manuel Blázquez-Duff [1], María Dolores del Castillo [2], Eugenio Miguel [1]

1 Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), N-II km 38,200, 28800 Alcalá de Henares, Spain.

2 Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), C/ Nicolás Cabrera, 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

Abstract

The aim of the present study was to evaluate the use of wine-making byproduct extracts (grape pomace, seed and skin) and dietary fiber (inulin and FOS) as suitable ingredients for the development of yogurts with antioxidant and antidiabetic properties. Their effect on the physicochemical, textural, microbiological and sensory parameters of yogurts was evaluated during 21 days of refrigerated storage. The incorporation of wine-making byproduct extracts in yogurt resulted in a significant increase in total phenolic content (TPC) and antioxidant and antidiabetic properties compared to the control. The grape skin yogurt showed the highest TPC (0.09 ± 0.00 mg GAE/g yogurt), antioxidant capacity (7.69 ± 1.15 mmol TE/g yogurt) and α-glucosidase inhibition (56.46 ± 2.31 %). The addition of dietary fiber did not substantially modify the overall antioxidant capacity or inhibition of the enzyme α-glucosidase of control and wine-making yogurts. Yogurts containing wine-making byproduct extracts and dietary fiber achieved high overall acceptance scores (6.33-6.67) and showed stable physicochemical, textural and microbiological characteristics during storage, assuring an optimal 21-day shelf life. Therefore, the use of wine-making byproduct extracts together with dietary fiber as ingredients in yogurt manufacture stand as a first approach towards the development of foods for sustainable health.

Keywords: carbohydrate metabolism, dietary fiber, functional yogurts, sustainable health, wine-making byproducts.

1. Introduction

Sustainable health is defined as the promotion of healthy ageing by preventing the risk of diseases [1]. Diet-related cardiometabolic diseases such as coronary heart disease, stroke, type 2 diabetes and obesity pose a substantial health and economic burden, causing 17 million deaths worldwide [2]. Consequently, there is an increasing demand for foods that promote sustainable health and wellbeing.

Different strategies for the promotion of healthier foods include product reformulation with a reduction of critical nutrients, such as added sugars, sodium and saturated fatty acids. Sweetened dairy products stand as one of the major food categories to focus action on due to their excessive amount of added sugars [3]. The incorporation of dietary fiber in yogurt may help replace sugar content as certain dietary fibers, such as inulin and fructo-oligosaccharides (FOS), have shown sweetening properties [4]. Moreover, intake of FOS and inulin provides many health benefits, including reduced incidence of diabetes and obesity due to their influence on appetite and energy intake through various mechanisms, including production of short-chain fatty acids (SCFA) due to colonic fermentation and subsequent regulation of gut hormones [5]. In addition, the incorporation of FOS nd inulin in yogurt may help consumers reach the 25 g of dietary fiber per day recommendation established by the European Food Safety Authority (EFSA). Similarly, food fortification with bioactive compounds, such as polyphenols, is another strategy for the incorporation of health-promoting ingredients in the human diet.

The wine-making industry produces a vast amount of byproducts which are well known for their antioxidant and antidiabetic properties [6]. The use of wine-making byproducts as ingredients in foods increases the sustainability of the food chain and could exert health-promoting effects through bioactivity beyond the basic nutrient composition. In this sense, the antidiabetic properties of the wine-making ingredients incorporated into a complex dairy matrix, such as yogurt, have not been previously studied. Therefore, the aim of this study was to evaluate the use of wine-making byproduct extracts (grape pomace, seed and skin) and dietary fiber (inulin and FOS) as suitable ingredients for the development of yogurts with antioxidant and antidiabetic properties. In addition, the effect of these ingredients on the physicochemical, textural, microbiological and sensory parameters of yogurts were evaluated during 21 days of cold storage.

2. Materials and methods

2.1. Raw materials

Ingredients used for yogurt preparation included UHT whole cow milk (Pascual, Spain), inulin Orafti®GR and fructo-oligosaccharides Orafti®P95 (Beneo, Belgium) and yogurt starter culture YO-MIX 300 (Danisco) containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus.

2.2. Extracts

Food grade commercial extracts from wine-making byproducts (grape pomace, skin and seed) were purchased from Natac (Spain). Grape pomace, seed and skin extracts were diluted in a Dimethyl Sulfoxide/ water mixture (1:10) at a final concentration of 1mg/mL and filtered with a 0.45 µm filter (Minisart Sterile16555). Samples were stored at -20ºC until composition and health-promoting activity analyses.

2.3. Yogurt samples

Eight yogurt formulations were prepared combining the three wine-making byproduct extracts with or without dietary fiber, a control with dietary fiber and no wine-making extracts, and a control without dietary fiber and wine-making extracts. Yogurt samples were produced heating the milk up to 45ºC to inoculate the starter culture. Dietary fiber was added to improve texture (inulin) and as a sweetener to replace sugar (FOS). Inulin and FOS were added at 7 g/100 mL and 10 g/100 mL, respectively. Wine-making byproducts extracts were dissolved in water and added to a concentration of 5 mg/mL in the yogurt. In control yogurts, the corresponding amount of water was added without extract. Milk was stirred, separated into pots and incubated at 45ºC during 5 h, when pH reached approximately 4.5. Samples were stored at 4ºC until further analyses. Yogurts were prepared in triplicate in three independent sessions. On day 1, 7, 14 and 21, yogurt samples (10 g) were diluted with 10 mL of distilled water and filtered in two steps with whaltman paper followed by a 0.45 µm filter (Minisart Sterile16555). The filtered extracts were stored at -20ºC until composition, health-promoting and technological analyses.

2.4. Composition analyses

Folin-Ciocalteu adapted to a micromethod format was used for the analysis of TPC in wine-making extracts and yogurt samples [7]. A gallic acid calibration curve (0.01 – 1 mg/mL) was used for quantification. Measurements were performed in triplicate.

In yogurts, total protein was determined by the Kjeldahl method as defined in the Commission Regulation No. 152/2009. Total fat was calculated by gravimetry defined in Commission Regulation No. 152/2009. Fatty acid profile was obtained by gas

chromatography (Agilent 7820A GC System equipped with Flame Ionization Detector) analyses, calculated according to the ISO 12966-2:2017. Lactose was measured using the Lactose-D-galactose Assay Kit (Megazyme, Ireland).

2.5. Health-promoting properties

2.5.1. Antioxidant capacity

- ABTS assay

The ABTS decolorization assay was performed as described by Oki *et al*., (2006). Aqueous solutions of Trolox (0.15–2.0 mM) were used for calibration. Measurements were performed in triplicate.

- Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay was applied according to the method of Ou, Hampsch-Woodill and Prior, (2001) as modified by Dávalos, Bartolomé, & Gómez-Cordovés, (2005). Measurements were performed in triplicate.

2.5.2. Antidiabetic properties

α-glucosidase inhibition was analyzed as in Berthelot & Delmotte (1999) and Geddes & Taylor (1985) and modified by Martinez-Saez, Hochkogler, Somoza, & del Castillo, (2017). Results were expressed as the concentration causing 50% inhibition (IC50) for wine-making byproduct extracts and as percentage of α-glucosidase inhibition for yogurt samples. All measurements were performed in triplicate.

2.6. Technological and shelf life characterization

The technological characterization (physicochemical and microbiological parameters) of yogurts containing wine-making extracts and dietary fiber was used as an indicator of the general quality of the yogurt during a 21-day shelf life period.

2.6.1. Physicochemical parameters

Moisture content was determined as described in AOAC-925.10. pH was measured with a Hanna Instruments HI5521 pH meter. Titratable acidity was determined according to ISO:RM 2012 and expressed as g lactic acid/ 100 g of yogurt. Yogurt syneresis was calculated in percentage according to the following equation:

$$\text{Syneresis (\%)} = [\text{expelled whey (g)/ yogurt mass (g)}] \times 100$$

The textural parameters of yogurts were obtained in triplicate using a TA.XTplus Texture Analyzer and analyzed with the Exponent software (Stable Micro Systems, UK). A back-extrusion test was carried out using a cylindrical stainless-steel probe (35 mm diameter) which was pressed into a cylindrical container (50 mm in diameter, 50 mm high). The probe penetrated the sample to a depth of 10 mm at 1 mm/s. Firmness (N) and consistency (Ns) were calculated from the deformation curves.

2.6.2. Viability of starter bacteria

Bacterial counts were carried out in triplicate following colony count technique (ISO 7889- IDF 117). *L. bulgaricus* colonies were counted in Man Rogosa Sharpe (MRS) agar (Pronadisa) after aerobic incubation at 37ºC for 72h. *S. thermophilus* colonies were counted in M17 agar (Pronadisa) after aerobic incubation at 37ºC for 48 h. Results were expressed as log CFU/mL of yogurt.

2.7. Consumer analysis

A pilot consumer test was performed using untrained consumers (n = 30). Analysis was performed in panel booths conforming to international standards (ISO 8589:2007). Yogurts (30 mL) were coded with a three-digit number and presented in random order to prevent first order and flavor carryover effects. Consumers were asked to rate the appearance, odor, texture, flavor and overall acceptability of yogurts using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely).

2.8. Statistical analysis

Analyses were performed using a one-way ANOVA with Tukey's test for assessing differences between samples. A two-way repeated measures ANOVA with Tukey's test was used to analyze shelf life data in different time points. Calculations were performed using IBM SPSS Statistics version 24.

3. Results and discussion

3.1. Composition and health-promoting properties of wine-making byproduct extracts

TPC analyses showed that grape skin extract had a significantly greater phenol content ($p < 0.01$) than seed and pomace extracts (Table 1). The presence of phenolic compounds in wine byproducts has been associated with antioxidant and antidiabetic properties. The antioxidant capacity of skin and seed extracts was significantly higher ($p < 0.001$) than that of grape pomace measured by ABTS. These results are in agreement with Felix da Silva *et al*. (2015).

Inhibition of α-glucosidase activity of grape skin and seed extracts was significantly higher ($p < 0.001$) than that of grape pomace (Table 1, Figure 1). IC50 of grape skin extract (0.30 ± 0.03 mg/mL) was similar to previously reported values from the Norton variety (0.38 mg/mL) [6]. However, IC50 of grape pomace (0.55 ± 0.06 mg/mL) and seed (0.36 ± 0.06 mg/mL) extracts were lower than those previously reported in red wine grape pomace of Cabernet Franc variety (1.63 mg/mL) [15] and muscadine seed extracts (1.53 mg/mL) [16]. This divergence may be due to differences in extraction methods, grape varieties and methods for assessing α-glucosidase inhibition. Previous studies have associated the inhibition of the enzymatic α-glucosidase activity of grapes with the presence of quercetin and ellagic acid in seeds, and anthocyanins and catechins from the skin and grape pomace [6, 17]. Overall, wine-byproducts showed strong α-glucosidase inhibition, which was higher than that reported in other natural inhibitors such as green tea (IC50 = 2.04 mg/mL), oolong tea (IC50 = 2.33 mg/mL) or black tea (IC50 = 2.73 mg/mL) [18].

Table 1. TPC, antioxidant capacity and antidiabetic properties of wine-making byproduct extracts.

	Grape Pomace	**Seed**	**Skin**
TPC (mg GAE/g extract)	278.07 ± 113.01[a]	437.50 ± 4.23[b]	502.04 ± 27.06[c]
Antioxidant capacity			
ABTS (mmol TE/g extract)	4.64 ± 0.17[a]	8.68 ± 0.51[b]	9.10 ± 0.50[b]
ORAC (mmol TE/g extract)	6.28 ± 0.74[a]	7.32 ± 0.48[a]	11.22 ± 0.76[b]
Antidiabetic properties			
α-Glucosidase inhibition (IC50 mg/mL)	0.55 ± 0.06[b]	0.36 ± 0.06[a]	0.30 ± 0.03[a]

Values represent mean ± standard deviation. Different letters are significantly different at $p < 0.01$

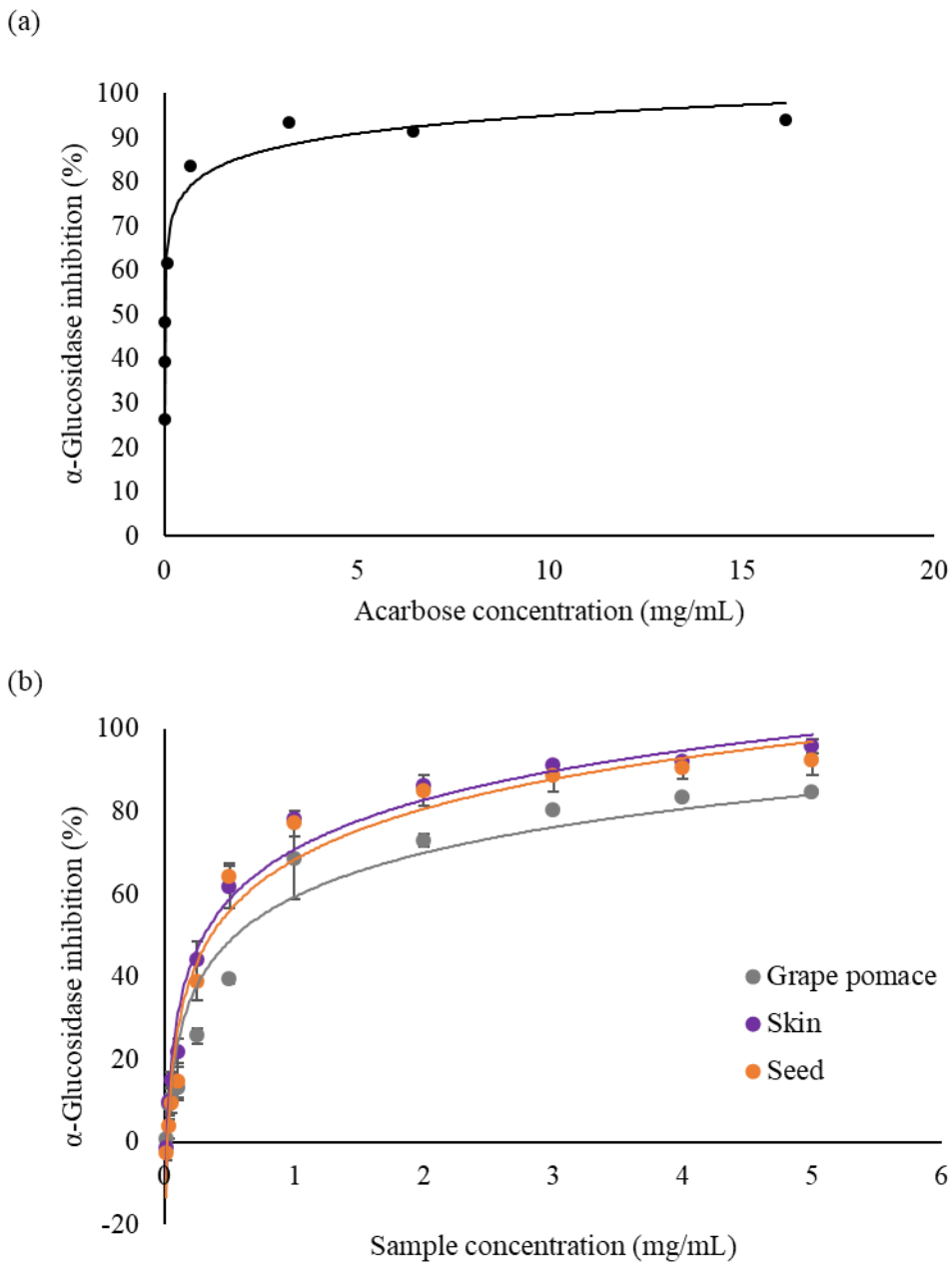


Fig. 1 Effect on α-glucosidase activity is represented by dose-response curves of (a) acarbose (0.06 µg/mL – 25 mg/mL); (b) Grape pomace, skin and seed extracts from wine-making byproducts (0.01 – 5 mg/mL). Values represent mean ± standard deviation. This includes a duplicate of sample preparation and a triplicate of analysis.

3.2. Application of wine-byproducts , FOS and inulin in yogurt

3.2.1. Yogurt composition

TPC of control yogurts significantly increased with the addition of wine-making extracts ($p < 0.001$) (Table 2). The grape skin yogurt showed a significantly higher TPC than the grape pomace and seed yogurts ($p < 0.001$). The addition of FOS and inulin did not modify the TPC in control and wine-making yogurts ($p > 0.05$).

Total lactose, protein and fat content were not affected ($p > 0.05$) by the addition of wine-making extracts (Table 3). Furthermore, non-significant differences ($p > 0.05$) were found in the fatty acid profile between yogurt formulations. Levels of individual fatty acids were in accordance with previous analyses on the fatty acid composition of

Table 2. TPC, antioxidant capacity and antidiabetic properties of yogurts containing wine-making byproduct extracts with or without dietary fiber.

	Wine-making byproduct extracts				***Wine-making byproduct extracts + dietary fiber***			
	Control	***Grape Pomace***	***Seed***	***Skin***	***Control***	***Grape Pomace***	***Seed***	***Skin***
TPC (mg GAE/g yogurt)	0.03 ± 0.00[a]	0.06 ± 0.00[b]	0.07 ± 0.01[b]	0.09 ± 0.00[c]	0.04 ± 0.00[a]	0.07 ± 0.01[b]	0.07 ± 0.00[b]	0.09 ± 0.00[c]
Antioxidant capacity								
ABTS (mmol TE/g yogurt)	0.31 ± 0.01[a]	0.97 ± 0.06[b]	1.16 ± 0.10[bc]	1.49 ± 0.08[d]	0.28 ± 0.01[a]	0.95 ± 0.07[b]	1.04 ± 0.07[b]	1.37 ± 0.10[cd]
ORAC (mmol TE/g yogurt)	4.32 ± 0.36[a]	5.50 ± 0.30[ab]	6.34 ± 1.10[ab]	7.69 ± 1.15[b]	4.19 ± 1.23[a]	6.24 ± 1.09[ab]	5.60 ± 1.35[ab]	7.84 ± 1.07[b]
Antidiabetic properties								
α-Glucosidase inhibition (%)	31.61 ± 3.26[a]	50.92 ± 1.70[b]	38.52 ± 5.87[a]	56.46 ± 2.31[b]	33.45 ± 3.35[a]	51.58 ± 1.15[b]	38.89 ± 1.34[a]	53.05 ± 0.44[b]

Values represent mean ± standard deviation. Different letters are significantly different at $p < 0.05$

α-Glucosidase inhibition (%) at yogurt concentration of 4 g/mL

cow yogurts [19]. Palmitic acid (C16:0) was the most predominant fatty acid, followed by oleic acid (C18:1n9c), myristic acid (C14:0) and stearic acid (C18:0).

Table 3. Fatty acid content (g/100 g of FA methyl esters) of functional yogurts enriched with grape pomace, seed and skin extracts at day 1 after yogurt manufacture.

Fatty acid	Control	Grape Pomace	Seed	Skin
Lactose (%)	3.24 ± 0.08	3.20 ± 0.2	3.10 ± 0.19	3.35 ± 0.51
Total Protein (%)	2.78 ± 0.07	2.72 ± 0.10	2.69 ± 0.02	2.67 ± 0.08
Total Fat (%)	2.88 ± 0.18	2.73 ± 0.24	2.70 ± 0.22	2.63 ± 0.18
Fatty acid profile (g/100 g FA methyl esters)				
C6:0	0.99 ± 0.02	0.96 ± 0.05	0.97 ± 0.02	0.97 ± 0.03
C8:0	1.03 ± 0.01	1.02 ± 0.05	1.01 ± 0.04	1.00 ± 0.04
C10:0	2.84 ± 0.10	2.81 ± 0.06	2.81 ± 0.09	2.81 ± 0.11
C11:0	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00
C12:0	3.59 ± 0.11	3.55 ± 0.08	3.54 ± 0.06	3.56 ± 0.15
C14:0	12.18 ± 0.05	12.10 ± 0.09	12.09 ± 0.06	12.14 ± 0.14
C14:1n5	1.13 ± 0.03	1.13 ± 0.05	1.13 ± 0.05	1.12 ± 0.04
C15:0	1.33 ± 0.07	1.33 ± 0.08	1.34 ± 0.08	1.33 ± 0.06
C16:0	35.86 ± 0.39	35.95 ± 0.08	35.95 ± 0.50	36.03 ± 0.31
C16:1n7	1.82 ± 0.07	1.84 ± 0.09	1.82 ± 0.07	1.83 ± 0.07
C17:0	0.62 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.62 ± 0.03
C18:0	9.92 ± 0.27	10.08 ± 0.55	10.08 ± 0.42	10.07 ± 0.32
C18:1n7c	1.12 ± 0.05	0.97 ± 0.26	1.01 ± 0.25	1.02 ± 0.13
C18:1n9c	21.52 ± 0.07	21.72 ± 0.33	21.69 ± 0.09	21.70 ± 0.26
C18:2n6c	2.44 ± 0.09	2.54 ± 0.09	2.46 ± 0.12	2.47 ± 0.10
C18:2n6t	0.23 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.23 ± 0.01
C18:3n3	0.46 ± 0.04	0.46 ± 0.03	0.45 ± 0.04	0.46 ± 0.02
C18:3n6	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.01
C20:0	0.15 ± 0.01	0.16 ± 0.03	0.14 ± 0.01	0.15 ± 0.01

C20:1n9	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00
C20:4n6	0.20 ± 0.02	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.00
C20:5n3	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00
C21:0	0.15 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.15 ± 0.00
C22:0	0.06 ± 0.00	0.06 ± 0.02	0.06 ± 0.00	0.05 ± 0.01
C22:5n3	0.07 ± 0.00	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.00
CLA	0.45 ± 0.01	0.47 ± 0.03	0.48 ± 0.02	0.47 ± 0.01
SFA	68.78 ± 0.08	68.88 ± 0.56	68.85 ± 0.31	68.97 ± 0.35
MUFA	25.67 ± 0.07	25.73 ± 0.05	25.73 ± 0.23	25.74 ± 0.31
PUFA	3.95 ± 0.16	4.07 ± 0.10	4.00 ± 0.17	4.00 ± 0.13

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Results are expressed as mean ± SD (n = 3).

At present there is great interest in specific fatty acids, such as conjugated linoleic acid (CLA), as its consumption has been associated with reduced carcinogenesis, atherosclerosis, body fat content and an improvement of hyperinsulinemia [20]. The CLA content of the yogurts containing wine-making extracts, FOS and inulin (0.45–0.48 %) was in accordance with those previously reported in cow milk yogurts (0.24–0.45 %) [19].

The presence of inulin and FOS in yogurts could potentially add further health-promoting benefits due to their prebiotic function. Inulin and FOS are resistant to digestion in the upper part of the intestinal tract and are subsequently fermented in the colon. The resulting SCFA, such as butyrate and propionate, have been associated with reduced risk of developing gastrointestinal disorders and cardiovascular disease [21].

3.2.2. Health-promoting properties

Yogurts containing wine-making extracts also showed a significant increase ($p < 0001$) in their antioxidant capacity. Grape skin yogurt had greater antioxidant capacity measured by ABTS ($p < 0.01$) than grape pomace and seed yogurts. However, grape pomace, seed and skin yogurts had similar antioxidant capacity measured by ORAC ($p > 0.05$). Adding dietary fiber did not substantially modify the antioxidant capacity of yogurts.

The overall antioxidant capacity observed in yogurts containing wine-making extracts was 93% lower than theoretically expected from the additive effects of bioactive

peptides in the control yogurt and wine-making byproduct extracts. Previous studies have also reported a significant decrease in the observed antioxidant capacity of yogurts with added polyphenol-rich fractions. The decrease in the observed antioxidant capacity was associated with a matrix effect due to the association between milk proteins and polyphenols from the byproduct extracts [22].

Inhibition of α-glucosidase activity was significantly higher ($p < 0.001$) in yogurts containing grape skin and grape pomace extracts compared to the control. Adding FOS and inulin did not significantly modify inhibition values ($p > 0.05$). The high inhibition of α-glucosidase activity by grape seed extract (IC50 = 0.36 mg/mL) did not result in higher enzymatic inhibition when the extract was added to the yogurt ($p > 0.05$). Previous studies have reported lower inhibition of α-glucosidase activity than expected in tomato puree and bread fortified with grape skin extracts [23]. This decrease could be attributed to molecular interactions between polyphenols and other components in the food matrix, such as proteins.

Inulin and FOS have also been associated with an improvement of glucose regulation by the modification of satiety hormone response (PYY and GLP-1) [24]. Therefore, the combined antidiabetic mechanisms of wine-making extracts and inulin and FOS may provide a novel approach to improve glucose metabolism. To our knowledge, this is the first time that the *in vitro* inhibition of the enzymatic activity of α-glucosidase has been determined in yogurts containing wine-making byproduct extracts with dietary fiber.

3.3. Technological parameters and shelf life characterization

Characterization of pH, titratable acidity, moisture, syneresis, viability of starter bacteria and instrumental texture during 21 days of cold storage is shown in Table 4. The addition of wine-making extracts did not significantly ($p > 0.05$) modify the yogurt's pH or titratable acidity, which is in accordance with Chouchouli *et al*. (2013). However, other studies have found that the addition of grape byproducts to yogurt significantly lowered its pH [26–28]. Discrepancies between results may be due to differences in yogurt formulations, such as the quantity and intrinsic pH value of the grape extract or flour used [27], the metabolic activity of certain probiotic strains [29] and the interaction with other ingredients [28].

Wine-making extracts had a significant effect on yogurt syneresis. On day 1, yogurt syneresis was significantly higher ($p < 0.05$) in the control (47.59%) than in yogurts with

Table 4. Evaluation of the shelf life stability of functional yogurts formulated with grape pomace, seed and skin extracts during 21 days of cold storage: physicochemical parameters, viability of starter bacteria and instrumental texture analysis.

	Days	Control	Grape Pomace	Seed	Skin	Significance
pH	1	4.75 ± 0.05^{C}	4.59 ± 0.07^{C}	4.60 ± 0.12^{C}	4.68 ± 0.05^{C}	ns
	7	4.39 ± 0.05^{B}	4.37 ± 0.08^{B}	4.44 ± 0.03^{B}	4.42 ± 0.04^{B}	ns
	14	4.32 ± 0.04^{AB}	4.33 ± 0.08^{AB}	4.37 ± 0.03^{AB}	4.32 ± 0.04^{AB}	ns
	21	4.31 ± 0.03^{A}	4.29 ± 0.05^{A}	4.35 ± 0.04^{A}	4.35 ± 0.04^{A}	ns
	Significance	***	***	***	***	
Titratable acidity (g lactic acid/ g yogurt)	1	0.57 ± 0.08^{A}	0.63 ± 0.04^{A}	0.62 ± 0.05^{A}	0.57 ± 0.05^{A}	ns
	7	0.67 ± 0.05^{B}	0.70 ± 0.03^{B}	0.67 ± 0.05^{B}	0.67 ± 0.05^{B}	ns
	14	0.74 ± 0.03^{C}	0.72 ± 0.03^{C}	0.71 ± 0.03^{C}	0.73 ± 0.01^{C}	ns
	21	0.76 ± 0.03^{C}	0.78 ± 0.04^{C}	0.75 ± 0.03^{C}	0.74 ± 0.03^{C}	ns
	Significance	***	***	***	***	
Moisture (%)	1	74.90 ± 0.31^{a}	75.53 ± 0.54^{ab}	76.24 ± 0.52^{ab}	76.50 ± 0.59^{b}	*
	7	75.34 ± 0.47^{a}	76.24 ± 0.52^{ab}	76.31 ± 0.39^{ab}	76.70 ± 0.36^{b}	*
	14	75.47 ± 0.47	76.27 ± 0.53	76.50 ± 0.96	76.56 ± 0.70	ns
	21	75.12 ± 0.44^{a}	76.02 ± 0.76^{ab}	76.86 ± 0.49^{b}	76.64 ± 0.34^{b}	*
	Significance	ns	ns	ns	ns	
Syneresis (%)	1	47.59 ± 4.38^{Bb}	30.75 ± 2.34^{Aa}	42.59 ± 5.26^{Aab}	34.57 ± 4.18^{Aa}	*
	7	43.89 ± 6.69^{B}	35.23 ± 3.09^{A}	41.94 ± 7.73^{A}	34.94 ± 2.65^{A}	ns
	14	40.72 ± 7.50^{A}	49.17 ± 4.16^{B}	48.94 ± 3.87^{B}	46.32 ± 5.18^{B}	ns
	21	40.77 ± 5.45^{A}	49.15 ± 4.31^{B}	50.13 ± 2.54^{B}	51.59 ± 4.34^{B}	ns
	Significance	*	*	*	*	

	Days	Control	Grape Pomace	Seed	Skin	Significance
***L. delbrueckii subsp. bulgaricus* (log cfu/mL)**	1	5.56 ± 0.19^{B}	5.65 ± 0.22^{B}	5.89 ± 0.20^{B}	5.75 ± 0.12^{B}	ns
	7	5.57 ± 0.18^{B}	5.65 ± 0.22^{B}	5.84 ± 0.25^{B}	5.81 ± 0.11^{B}	ns
	14	5.43 ± 0.27^{AB}	5.49 ± 0.18^{AB}	5.60 ± 0.22^{AB}	5.29 ± 0.59^{AB}	ns
	21	5.10 ± 0.21^{A}	5.11 ± 0.33^{A}	4.96 ± 0.42^{A}	5.02 ± 0.19^{A}	ns
	Significance	***	***	***	***	
***S. thermophilus* (log cfu/mL)**	1	9.07 ± 0.05	9.14 ± 0.07	9.05 ± 0.09	9.18 ± 0.18	ns
	7	9.28 ± 0.08	9.29 ± 0.29	9.00 ± 0.26	9.11 ± 0.16	ns
	14	9.11 ± 0.01	9.00 ± 0.14	8.98 ± 0.04	8.94 ± 0.12	ns
	21	9.00 ± 0.17	9.16 ± 0.08	9.03 ± 0.19	9.02 ± 0.02	ns
	Significance	ns	ns	ns	ns	
Firmess (N)	1	1.88 ± 0.44	2.66 ± 0.51	2.79 ± 0.23	2.59 ± 0.91	ns
	7	2.25 ± 0.15	2.99 ± 0.14	2.70 ± 0.36	2.93 ± 0.24	ns
	14	2.63 ± 0.22	2.71 ± 0.20	2.90 ± 0.52	3.01 ± 0.56	ns
	21	2.30 ± 0.34	2.69 ± 0.67	2.92 ± 0.96	3.04 ± 0.47	ns
	Significance	ns	ns	ns	ns	
Consistency (Ns)	1	16.3 ± 4.21	24.82 ± 5.21	25.12 ± 3.71	24.08 ± 8.87	ns
	7	20.04 ± 1.05	26.69 ± 2.88	23.23 ± 6.04	26.92 ± 3.39	ns
	14	24.19 ± 1.94	23.74 ± 3.95	26.17 ± 4.93	24.23 ± 2.77	ns
	21	20.87 ± 2.25	22.69 ± 5.52	27.11 ± 9.17	26.95 ± 4.1	ns
	Significance	ns	ns	ns	ns	

Superscript uppercase letters in each column indicate statistically significant differences during storage. Superscript lowercase letters in each row indicate statistically significant differences between yogurt samples. * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns not significant

added grape pomace and skin extracts (30.75% and 34.57%, respectively) (Table 4). However, an inverted pattern of whey release was found during storage. While the control treatment with FOS and inulin significantly decreased syneresis ($p < 0.05$) during storage, yogurts with wine-making extracts, FOS and inulin had significantly greater syneresis ($p < 0.05$) during the last two weeks of storage. Increased syneresis during storage was also observed in yogurt fortified with grape pomace flour [27]. According to the protein-polyphenol interaction model [30], the high syneresis values observed in yogurts fortified with wine-making extracts could be due to great amount of polyphenols present.

According to Tseng & Zhao (2013), the timing of addition of the polyphenol-rich extracts can also affect yogurt syneresis. They observed greater syneresis rates when the extracts were added to milk before fermentation than when added once the yogurt gel was formed. This could also explain the high syneresis values obtained in our study, as the wine-making extracts were added before milk fermentation.

Moisture content was significantly higher ($p < 0.05$) in grape skin yogurt than in the control during storage. This was contrary to our expectations, as addition of the extracts increased the percentage of dry matter, reducing the amount of moisture. This deviant result in grape skin extracts could be due to the different interactions between grape skin polyphenols and other matrix components.

The instrumental texture parameters of functional yogurts are shown in Table 4. A slight tendency of wine-making extracts increasing texture parameters of yogurts containing dietary fiber was observed, but differences were not significant ($p > 0.05$). This may be due to the high variability of the texture analysis and low reproducibility of yogurt elaboration. The high firmness and consistency values of yogurts were similar to those of Greek yogurt, probably due to the texturizing properties of inulin [4, 31]. Regarding shelf life, previous studies have observed a significant increase in the firmness and consistency of yogurts containing grape pomace extract after 28 days of cold storage [29]. Although no significant differences ($p > 0.05$) were observed in the present study during storage, firmness and consistency tended to increase slightly with time.

Results of the microbiological analysis are shown in Table 4. Bacterial counts in the control and wine-making yogurts throughout their shelf life were higher than the minimum of 7 log CFU/mL legally required in yogurt manufacture by the Codex Alimentarius. The initial and final S. thermophilus counts (9.05-9.18 and 9.00-9.16 log CFU/mL, respectively) were significantly higher ($p < 0.001$) than those obtained for *L. delbrueckii subsp. bulgaricus* (5.56–5.89 and 4.96-5.11 log CFU/mL, respectively).

Lower counts of *L. delbrueckii subsp. bulgaricus* than *S. thermophilus* is a common observation in yogurt elaboration with commercial cultures of bacteria, which may be a strategy to minimize the acetic acid taste produced from the metabolism of *L. delbrueckii subsp. bulgaricus* [32].

The addition of wine-making extracts to yogurt did not significantly affect ($p > 0.05$) bacterial counts during storage, suggesting that the phenolic compounds of wine-making extracts did not affect the viability of starter bacteria. However, further studies would be needed to analyze the effect of the wine-making extracts on the yogurt's fermentation kinetics. The number of viable *S. thermophilus* remained constant during the storage period of all yogurt samples, whereas *L. delbrueckii subsp. bulgaricus* counts significantly decreased after 21 days of storage. These results are in accordance with previous studies which used L. delbrueckii subsp. bulgaricus and S. thermophilus in the development of yogurts containing grape marc preparations [26, 33].

3.4. Pilot consumer test

Consumers scored all yogurt formulations similarly ($p > 0.05$) in terms of smell, flavor, texture and overall acceptability (Table 5). Significant differences were only observed in appearance, which was higher in the control and grape skin yogurts ($p < 0.05$) (6.96 and 7.30, respectively) than in the grape pomace and seed yogurts (5.96 and 5.93, respectively). Consumers may have recognized the control as a conventional yogurt and its familiarity could explain its high visual acceptability. The novel food color provided by the grape skin extract was also highly accepted, suggesting that color innovations in a purple palette for yogurts could be marketable. The influence of color on food preference has been extensively studied. Food coloring can influence flavor identification, perception and preferences, and can even dominate other flavor sources of information, such as labelling and taste [34]. As the consumer test was conducted in blind conditions (no information on the composition of yogurts was provided to volunteers), the color-flavor associations made by consumers were unknown.

In general, yogurt formulations obtained high overall liking scores, which ranged between 6.3 and 6.7 on a 9-point hedonic scale. However, several studies have reported a negative correlation between wine-making byproduct concentrations and overall yogurt acceptance [27, 28]. Studies in which wine-making byproducts were added together with other ingredients significantly improved yogurt formulation. The addition of 5% sucrose [33], grape juice and sucrose [29] or a combination of oligofructose and purple grape juice [28] improved the overall acceptance of yogurts containing grape pomace and grape skin preparations. In this sense, we used inulin and FOS to improve

the sensory properties and meet the desired functional fiber enrichment. Inulin has previously been described to improve yogurt texture and mouthfeel [4]. FOS are more soluble and sweeter than inulin, and can also improve mouthfeel [35]. The sweetening power (30-35%) and low caloric content (1-2 kcal/g) of FOS justified its use as a sucrose replacer. The dietary fiber content of the yogurts would also allow a "high in fiber" nutritional claim on the package. In this sense, an 80 g portion of the formulated yogurts would provide half the dietary fiber daily intake recommended by the EFSA.

Table 5. Pilot consumer analysis (n = 30) of yogurts containing wine-making byproduct extracts and control yogurt.

	Control	Grape pomace	Seed	Skin
				
Appearance	6.96 ± 1.13[b]	5.96 ± 1.34[a]	5.93 ± 1.49[a]	7.30 ± 1.30[b]
Smell	6.30 ± 1.23[a]	5.96 ± 1.32[a]	5.44 ± 1.25[a]	5.78 ± 1.40[a]
Taste	6.67 ± 1.39[a]	6.52 ± 1.60[a]	6.48 ± 1.63[a]	5.89 ± 1.99[a]
Texture	7.07 ± 1.44[a]	7.04 ± 1.43[a]	6.74 ± 1.43[a]	6.85 ± 1.35[a]
Overall Acceptability	6.67 ± 1.36[a]	6.33 ± 1.52[a]	6.33 ± 1.41[a]	6.37 ± 1.64[a]

Data are expressed as mean ± standard deviation. Different letters indicate significant differences between yogurt samples ($p < 0.05$).

4. Conclusion

This study showed that grape pomace, seed and skin byproduct extracts can be used together with inulin and FOS in the development of yogurt as sources of bioactive compounds and dietary fiber. Adding FOS and inulin did not substantially modify the potential biological benefits of the yogurts, which presented high antioxidant and antidiabetic properties. Moreover, the dietary fiber content of the yogurts would allow the products to have the "high in fiber" nutritional claim. The grape skin yogurt presented the highest overall antioxidant capacity and inhibition of α-glucosidase activity. In addition, the grape skin yogurt's appearance achieved the best acceptance score, suggesting that the grape skin extract could have a potential use as a colorant in dairy products. Therefore, the results of this study show that wine-making byproduct extracts can be combined with FOS and inulin for the development of health-promoting and sustainable yogurts.

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Study 2: The sustainability effect: consumers' perception on the use of byproducts from the wine industry as novel ingredients in yogurt.

Iriondo-DeHond, M., Varela, P., del Castillo, M.D., & Miguel, E. 2019. The sustainability effect: consumers' perception on the use of byproducts from the wine industry as novel ingredients in yogurt.

The sustainability effect: consumers' perception on the use of byproducts from the wine industry as novel ingredients in yogurt

Maite Iriondo-DeHond [1,2], Paula Varela [3], María Dolores del Castillo [2], Eugenio Miguel [1]

[1] Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), N-II km 38,200, 28800 Alcalá de Henares, Spain.

[2] Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), C/ Nicolás Cabrera, 9, Universidad Autónoma de Madrid, 28049 Madrid, Spain.

[3] Nofima AS. P.O. Box 210, 1431 Ås, Norway.

Abstract

The aim of the present study was to evaluate consumers' perception on the use of byproducts from the wine industry as novel food ingredients in functional yogurts. Three yogurt samples containing grape pomace, grape seed and grape skin extracts were evaluated by 100 consumers who scored the overall liking under blind, expected and informed conditions. Consumers were also asked to answer a check-all-that-apply questionnaire concerning sensory and non-sensory terms. In addition, an "ideal grape yogurt" was included in the CATA question to perform a penalty analysis to show potential directions for product reformulation. Results showed that overall liking was influenced by the type of byproduct ingredient, the evaluation condition and its interaction. Yogurt formulations improved their overall liking under informed conditions compared to the blind test, suggesting that information on origin of the byproducts exerted a positive influence on consumers' perception on the product. Expected liking varied greatly among samples. The hedonic expectation raised by the grape skin yogurt was significantly higher ($p < 0.05$) than in the rest of the yogurt formulations, whereas the grape seed yogurt obtained the lowest expected liking scores. Product information on expected and informed conditions led consumers to describe yogurts containing wine-making byproduct extracts as "novel", "interesting", "sustainable", "antioxidant" and "healthy", suggesting that yogurt ingredients were associated with both sustainable and health concepts. Penalty analysis showed a clear direction for product reformulation towards a more pleasant and sweet taste. Overall, results suggest that consumers have a positive perception on the use of wine-making byproducts as novel ingredients in yogurts, which is an important consideration for the yogurts commercial feasibility.

1. Introduction

The current global food system is facing the challenge of delivering food to a fast growing population while helping to achieve environmental sustainability [1]. It has been estimated that worldwide food losses and wastes (FLW) represent one third of the food produced for human consumption in mass, which is equivalent to 1.3 billion tons per year [2]. Therefore, national and international institutions have developed specific implementation plans, such as the European Bioeconomy Strategy 2030 or the North American Initiative on Food Waste Reduction and Recovery, to prioritize their reduction and, subsequently, improve food security and the environmental footprint of food systems [3]. The conversion of food byproducts into high added-value ingredients aimed at human consumption is currently one of the leading strategies for FLW reduction. This is because food byproducts represent a promising source of functional compounds, which have been associated with potentially beneficial biological and nutritional properties for human health [4–6].

Sustainability has become an additional factor to be considered among consumers' food choice motives and is currently associated with a positive conceptualization. Interestingly, although consumers are aware of the sustainability concept, they are not able to define it precisely, indicating that sustainability is a indiscriminate issue on consumers' minds [7]. This may be due to the multidimensional notion of sustainability, which englobes environmental, social and economic system considerations [8]. In addition, consumers are increasingly exposed to information on different environmental threats (population growth and urbanization, energy use and global warming, water scarcity and waste generation, pollution of soil, air, and water, limited supply of resources, etc.) and sustainable trends (animal welfare, clean-label, local production, organic foods, plastic-free packaging, etc.), which may contribute to their confusion. In this sense, different approaches have been explored to measure the weight of sustainability on consumers' food choice pattern, including measurements of ethical food choice motives [9], sustainable product choices and curtailment behavior [10], or studies on the influence of certain types of information, such as organic food origin or carbon footprint, on the hedonic expectation of products [7,11,12].

The valorization of food byproducts as novel ingredients for the development of functional foods is an approach that has been implemented more and more frequently over the last years. Namely, dairy products have been one of the food sectors where many of these innovations have focused, probably due to dairy foods standing as the preferred matrix for the vehiculization of bioactive compounds [13,14]. Wine-making

byproducts, including grape pomace, skin and seeds, whose annual generation is estimated in 14.5 million tons in Europe alone [15], represent one of the byproduct groups most widely applied in potentially functional yogurts and fermented milks [16]. The organoleptic profile, overall liking and information regarding potential product reformulation of yogurts and fermented milks containing wine-making byproducts have been evaluated [17–21]. However, information on consumer perception of food byproducts as novel ingredients in these novel formulations is still missing, leaving the commercial feasibility of such foods questionable.

To our knowledge, only one previous article has studied consumer's perception towards foods containing byproducts, which focused on the extrinsic cues of the food products [22]. This way, the present study stands as a first attempt to evaluate consumers' perception of using food byproducts as novel ingredients when combining extrinsic and intrinsic product cues. The aim of the present study was to evaluate how information on the incorporation of different wine-making byproducts (grape pomace, seed and skins) in yogurts may affect consumers' food acceptability and expectations. For this purpose, consumers' acceptance was explored in blind, expected and informed conditions.

2. Materials and methods

2.1. Yogurt samples

Three set-type yogurt formulations were prepared: grape pomace, grape skin and grape seed yogurts. Yogurt samples were made using whole UHT cow milk (3.6% fat, 3% protein, 4.8% sugar), starter culture YO-MIX 300 (Danisco), 6% (w/v) inulin (Orafti®GR, Beneo) and 3% (w/v) maltitol. Byproduct extracts were dissolved in water and added into the milk prior to fermentation at a concentration of 5 mg/mL. Fermentation was completed when yogurts reached a pH of 4.5 and were stored at 4 ºC overnight.

2.2. Participants

The study was conducted in Madrid in two different locations recruiting a total of 100 participants from the surrounding areas of the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA) and Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC). Participants ranged in age from 18 to 60 including 63 women and 37 men. All participants were regular consumers of yogurt. The same consumers participated in the three evaluation conditions: blind, expected and informed, as explained in the following section. No compensation was given to consumers for their participation.

2.3. Experimental procedure

The experimental procedure involved the collection of data in three evaluation conditions: blind, expected and informed. In each evaluation condition the three samples (grape pomace, grape skin and grape seed yogurts) were given one at a time, following a Williams' Latin square experimental design balanced for first order carryover effects. Data were collected in paper questionnaires, which were explained to each consumer individually prior to the start of the session. In each condition, participants had to indicate their overall or expected liking and answer a CATA (check all that apply) questionnaire.

First, consumers evaluated the samples under blind conditions. Samples were served in 30 mL portions at refrigeration temperature (4º C) and were presented in three-digit codes for sample identification. In the expected condition, participants received a picture of each one of the yogurts with the information of the corresponding yogurt sample: "The image in front of you shows a high dietary fiber yogurt made with a natural extract of red grape pomace/ red grape skin/ grape seed recovered from the winemaking process. List of ingredients: milk, dietary fiber, maltitol (hypocaloric sweetener), grape pomace/ grape skin/ grape seed extract, dairy starter cultures". After the expected condition participants were instructed to fill out an attitudinal questionnaire. In the informed condition, participants received the previous information accompanied by the corresponding yogurt sample. Finally, participants were asked to answer to the same liking and CATA questionnaires regarding their ideal concept of a grape yogurt.

2.4. Acceptability test

Overall liking was assessed using a nine-point hedonic scale (9 = like extremely, 1 = dislike extremely). Overall liking was placed at the beginning of the questionnaire in each evaluation conditions.

2.5. CATA questionnaire

For each sample participants answered a CATA questionnaire featuring 32 terms. Participants were asked to check all the terms that applied to the corresponding sample. The terms had been previously selected from available literature and previous consumer studies. The final questionnaire included 18 sensory terms (acid, bitter, sweet, grape taste, wood taste, pleasant taste, unpleasant taste, herbaceous taste, insipid, milky, creamy, firm, grainy, soft texture, astringent, heterogeneous, homogeneous, pleasant color) and 14 non-sensory terms (novel, satiety, interesting, strange, unhealthy, high in

dietary fiber, sustainable, traditional, antioxidant, boring, healthy, artificial, natural and familiar). The order in which the 32 attributes were presented was randomized within the two groups (sensory and non-sensory), between products and among consumers.

2.6. Attitudinal questionnaire

After the expected condition participants were given a questionnaire regarding their attitudes towards health, sustainability and food novelty. The questionnaire included the items from the "Health" and the "Natural content" factors from the Food Choice Questionnaire (FCQ) [23] translated into Spanish according to Jáuregui-Lobera & Bolaños Ríos, (2011). Two items from the "Environmental protection" factor for measuring ethical food choice motives were also included [9]: it is important to me that the food I eat on a typical day (I) "has been prepared in an environmentally friendly way" and (II) "has been produced in a way which has not shaken the balance of nature". The following item specifically related to the use of byproducts as food ingredients was included and generated through author's discussion: "It is important to me that the food I eat on a typical day uses food byproducts from the food industry as sources of novel ingredients". The general attitude towards novelty in food was measured by the 10-item Food Neophobia (FN) scale [25]. Both FCQ and FN questions were answered on a 7-point likert scale anchored from "completely agree" to "completely disagree". The change from the original FCQ four-box scale was done with the purpose of increasing the discrimination ability among food choice motives. Additionally, an 8-item Variety Seeking (VS) scale was completed to measure the extent to which participants seek out food variety [26]. VS questions were answered in a 5-point likert scale anchored from "completely agree" to "completely disagree". The demographic variables of age, sex and education level were also included.

2.7. Data analyses

The study of the relation of liking scores between blind, expected and informed evaluation conditions was performed with a repeated measures analysis of variance (RM-ANOVA) considering yogurt sample and evaluation condition as two within-subject factors.

Pairwise comparisons between the means of the significant main and simple effects were performed to identify where differences occurred. Multiple comparisons were adjusted with the Bonferroni correction. Calculations were performed using IBM SPSS Statistics version 24.

Cochran's Q test was performed on each of the CATA question descriptors to detect differences in consumer perception of yogurt samples. A penalty analysis on the CATA data was performed to determine the drop in the global liking associated with the deviation of each product from the ideal for each attribute [27]. Penalty analysis was performed to identify "must have", "nice to have" and "must not have" attributes for obtaining directions for product reformulation [28]. "Must have attributes" are those in which the attribute is missing in the sample but is present in the ideal product. When an attribute is missing in the ideal but is present in the sample product it can lead to two situations: if the attribute present in the sample leads to an increase in liking it is defined as a "nice to have" attribute; but if it affects liking negatively, it is a "must not have" attribute. In this analysis mean drops in liking between the two situations are calculated and their significances are tested.

In order to identify groups of consumers who had different overall liking of the yogurt samples in the different evaluation conditions, an agglomerative hierarchical cluster analysis was performed on the overall liking scores in blind, expected and informed conditions separately. Euclidean distances and Ward aggregation method were considered. The existence of differences between the overall liking between consumer groups was evaluated by one factor ANOVA.

The Cochran's Q test, the penalty analyses and cluster analyses were performed using XLSTAT statistical software (version 2015, Addinsoft®, Barcelona, Spain).

3. Results and discussion

Analysis of variance by repeated measures showed that main effects from yogurt type ($F = 7.7, p < 0.001$), evaluation condition ($F = 6.2, p < 0.01$) and their interaction ($F = 12.2, p < 0.001$) were significant (Table 1, Figure 1). Therefore, the interpretation of results is explained in relation to the different levels of the evaluation condition.

3.1. Blind testing

Overall liking of the yogurt samples was significantly different under blind testing conditions (Table 1), indicating that consumers reacted differently to the sensory characteristics of the samples. The grape pomace yogurt showed a significantly greater liking score ($p < 0.01$) than the grape seed and skin yogurts. Overall liking scores ranged from 6.0 to 6.8, corresponding to products classified as slightly to moderately liked in the 9-point hedonic scale. These liking values are in the same range (6 – 7.5) as those

obtained using grape skin flour and grape pomace extracts in yogurts [19,20]. However, in these studies the addition of the winery byproduct ingredient was accompanied by the addition of sucrose or grape juice, which also has a high sucrose content. Sucrose addition is one of the main strategies for reducing the bitterness and astringency associated to polyphenol-rich ingredients [29]. In this sense, our yogurt formulations achieved similar overall liking scores to sugar-sweetened products using inulin and maltitol, which present a nutritional advantage. Inulin is a soluble non-digestible carbohydrate with prebiotic effect which has been associated with the prevention of diet-related chronic diseases [30,31]. Maltitol has a lower caloric intake than sugars (2.4 kcal vs 4 kcal per gram) and a reduced glycaemic response [32].

Table 1. Mean liking scores for the yogurt samples evaluated in blind, expected and informed conditions.

	Evaluation condition		
Sample	Blind	Expected	Informed
Grape pomace	6.8^{abB}	6.6^{aB}	7.0^{bB}
Seed	6.3^{aA}	6.2^{aA}	6.5^{aA}
Skin	6.0^{aA}	7.1^{cC}	6.5^{bAB}

Different lowercase letters indicate significant differences between evaluation conditions according to pairwise mean comparisons adjusted by Bonferroni ($p < 0.05$). Different capital letters indicate differences between yogurt samples according to pairwise mean comparisons adjusted by Bonferroni ($p < 0.05$).

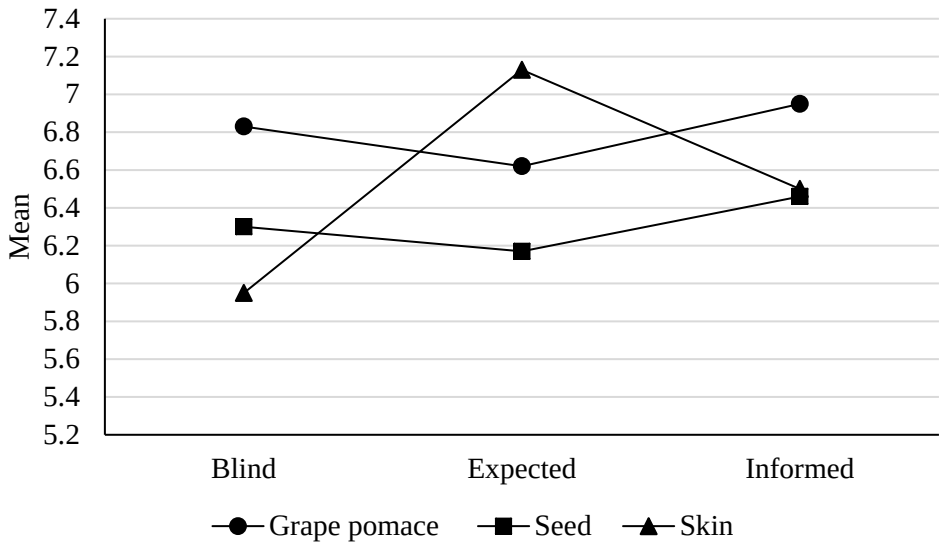

Figure 1. Interaction plot for overall liking between sample type (grape pomace, seed and skin) and evaluation condition (blind, expected and informed).

For each attribute in the CATA question, the number of consumers that checked the attribute for a particular sample is shown in Table 2. Cochran's Q test showed that frequencies varied significantly among yogurt samples in 7 out of the 18 sensory attributes, and in 5 out of 14 non-sensory terms. Significant differences were found in attributes related to the taste (*sweet, acid, disgusting taste, grape flavor* and *milky*) and color (*pleasant color*) of the samples, whereas no significant differences ($p > 0.05$) were observed among texture attributes except for *homogeneous*.

Grape skin yogurt was most frequently selected as *pleasant color, disgusting taste* and *grape flavor* and less frequently cited as *milky*. Although previous studies have shown a strong association between the color purple and the taste bitter [33], this attribute was not significantly more cited in the grape skin yogurt than in the rest of the yogurt samples. In relation to the non-sensory terms, the grape skin yogurt was most frequently selected as *antioxidant* and *strange*, and less selected as *traditional, familiar* and *natural* compared to the other yogurt formulations. These results suggest that the purple color of the skin yogurt was appealing and derived in associations with health-related concepts such as *antioxidant*. However, the organoleptic properties of the yogurt led to descriptions with negative connotation, such as *disgusting taste*. These results suggest that the influence of color may have given a first expectation that was not met after tasting the yogurt, which may explain the selection of the *strange* term.

Table 2. Number of consumers (n = 100) which used the terms of the CATA questions to describe the yogurt samples in the different evaluation conditions tested (blind, expected and informed) and their ideal product, and results from Cochran's Q test for comparison between samples.

		Blind conditions				***Expected conditions***				***Informed conditions***			
Attributes	IDEAL	significance	GPB	SB	SKB	significance	SKE	GPE	SE	significance	GPI	SKI	SI
pleasant color	94	***	49^{a}	47^{a}	74^{b}	***	80^{b}	62^{a}	50^{a}	***	57^{a}	83^{b}	55^{a}
sweet	65	*	29^{ab}	36^{b}	22^{a}	ns	38	42	33	ns	40	35	37
acid	28	**	32^{b}	17^{a}	20^{ab}	ns	34	31	24	ns	29	24	19
bitter	1	ns	7	5	14	ns	15	14	26	**	5^{a}	15^{b}	15^{b}
pleasant taste	92	ns	59	63	55	ns	58	52	46	ns	69	62	58
disgusting taste	0	***	1^{a}	3^{ab}	12^{b}	ns	2	2	8	**	1^{a}	11^{b}	4^{ab}
grape flavor	70	***	13^{a}	12^{a}	39^{b}	***	86^{c}	66^{b}	48^{a}	***	22^{a}	51^{b}	21^{a}
woody flavor	5	ns	6	16	11	***	4^{a}	11^{a}	25^{b}	***	9^{a}	12^{a}	25^{b}
Liking	6.7		6.8	6.3	6.0		7.1	6.6	6.2		7.0	6.5	6.5

Samples are arranged in descending texture liking order from left to right within each evaluation condition

*** Indicates significant differences between samples according to Cochran's Q test at $p < 0.001$.

** Indicates significant differences between samples according to Cochran's Q test at $p < 0.01$

* Indicates significant differences between samples according to Cochran's Q test at $p < 0.05$

ns Indicates no significant differences between samples according to Cochran's Q test ($p > 0.05$).

Table 2 (Continued)

		Blind conditions				***Expected conditions***				***Informed conditions***			
Attributes	IDEAL	significance	GPB	SB	SKB	significance	SKE	GPE	SE	significance	GPI	SKI	SI
herbal flavor	14	ns	8	10	10	ns	17	21	27	ns	10	20	20
insipid	1	ns	16	16	16	ns	3	7	12	ns	10	6	9
milky	18	**	30^{b}	31^{b}	16^{a}	**	15^{a}	25^{ab}	29^{b}	***	35^{b}	18^{a}	36^{b}
astringent	17	ns	24	32	26	**	33^{a}	40^{ab}	53^{b}	ns	31	34	36
firmness	46	ns	32	24	25	ns	31	28	26	**	33^{ab}	37^{b}	21^{a}
creamy	92	ns	74	69	71	ns	64	60	52	ns	76	71	73
soft	85	ns	80	78	76	ns	58	59	48	**	85^{b}	81^{ab}	69^{a}
grainy	1	ns	0	7	1	ns	10	8	15	**	2^{a}	5^{ab}	12^{b}
homogeneus	76	*	72^{b}	59^{a}	72^{b}	**	49^{ab}	59^{b}	43^{a}	***	70^{b}	73^{b}	52^{a}
heterogeneus	5	ns	2	10	4	ns	12	4	9	***	4^{ab}	0^{a}	14^{b}
Liking	6.7		6.8	6.3	6.0		7.1	6.6	6.2		7.0	6.5	6.5

Samples are arranged in descending texture liking order from left to right within each evaluation condition

*** Indicates significant differences between samples according to Cochran's Q test at $p < 0.001$.

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ns Indicates no significant differences between samples according to Cochran's Q test ($p > 0.05$).

Table 2 (Continued)

		Blind conditions				***Expected conditions***				***Informed conditions***			
Attributes	IDEAL	significance	GPB	SB	SKB	significance	SKE	GPE	SE	significance	GPI	SKI	SI
healthy	95	ns	34	27	26	**	80^{b}	63^{a}	72^{ab}	ns	66	69	65
not healthy	0	ns	0	1	0	ns	2	1	1	ns	1	1	1
antioxidant	78	***	7^{a}	8^{a}	29^{b}	**	72^{b}	58^{a}	54^{a}	***	50^{a}	65^{b}	47^{a}
high in fiber	84	ns	2	6	4	ns	84	77	87	ns	58	66	65
satiety	42	ns	12	14	11	ns	43	40	38	ns	30	35	36
sustainable	83	ns	2	4	5	ns	57	51	54	ns	51	53	50
natural	78	**	47^{b}	38^{ab}	27^{a}	ns	43	43	47	ns	51	42	41
artificial	1	ns	3	8	13	ns	13	9	7	ns	6	6	11
Liking	6.7		6.8	6.3	6.0		7.1	6.6	6.2		7.0	6.5	6.5

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ns Indicates no significant differences between samples according to Cochran's Q test ($p > 0.05$)

Table 2 (Continued)

		Blind conditions				***Expected conditions***				***Informed conditions***			
Attributes	IDEAL	significance	GPB	SB	SKB	significance	SKE	GPE	SE	significance	GPI	SKI	SI
novel	65	ns	21	21	36	ns	78	71	69	ns	66	73	62
familiar	26	*	24^{b}	16^{ab}	10^{a}	ns	8	11	9	*	14^{ab}	5^{a}	16^{b}
traditional	20	**	19^{b}	16b	4^{a}	ns	5	10	7	ns	13	5	10
interesting	73	ns	31	37	47	ns	65	55	60	ns	63	63	57
strange	7	***	9^{a}	16^{a}	32^{b}	ns	16	21	26	ns	16	24	26
bored	1	ns	7	9	6	ns	0	6	6	ns	5	3	6
Liking	6.7		6.8	6.3	6.0		7.1	6.6	6.2		7.0	6.5	6.5

Samples are arranged in descending texture liking order from left to right within each evaluation condition

*** Indicates significant differences between samples according to Cochran's Q test at $p < 0.001$.

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ns Indicates no significant differences between samples according to Cochran's Q test ($p > 0.05$)

Results showed that the sensory and non-sensory terms that showed a significant difference in the frequency of their selection in the grape skin yogurt implied a more negative connotation compared to the other yogurt formulations. However, the overall liking score of the product was still acceptable (6.0), most likely due to the pleasant color and antioxidant associations, which were significantly higher in this product ($p < 0.001$).

Penalty analysis on blind conditions identified "must have" sensory attributes for the three samples (Figure 2a). *Pleasant taste* was a "must have" attribute that had been selected in the ideal product but missing in the three yogurt samples. Missing this attribute affected overall liking 1.0, 1.7 and 2.6 points for the grape pomace, grape seed and grape skin yogurts, respectively. *Creamy* was another "must have" attribute for the grape skin and grape seed yogurts, which would significantly increase their overall liking by 1.3 and 1.0 points, respectively. Pleasant color and sweet were "must have" attributes for the seed yogurt. In relation to non-sensory attributes, *interesting* was found to be a "must have" attribute for the grape seed and skin yogurts, affecting on their overall liking by 1.3 and 2.3 points, respectively. *Healthy* and *natural* were two "must have" attributes highly selected in the ideal product but missing in the grape pomace and grape skin yogurts by 63% and 55% of the consumers, respectively.

Results from the cluster analysis on overall liking in blind conditions identified 3 consumer clusters, consisting of 26, 36 and 38 consumers (Table 3). The three clusters showed significant differences in the overall liking of the yogurt samples. The grape pomace yogurt was highly accepted by consumers from the three clusters (overall liking between 6.5 to 7.2). The grape seed yogurt received a high liking score (7.8) in Cluster 2, which was significantly different ($p < 0.05$) than the liking values from Clusters 1 and 3. Lastly, the grape skin yogurt obtained a significantly lower ($p < 0.05$) liking score than the grape pomace and seed yogurts. Cluster 2 rated all yogurts with liking scores over 7.0, suggesting that consumers from this cluster liked all yogurt formulations containing wine-making byproducts.

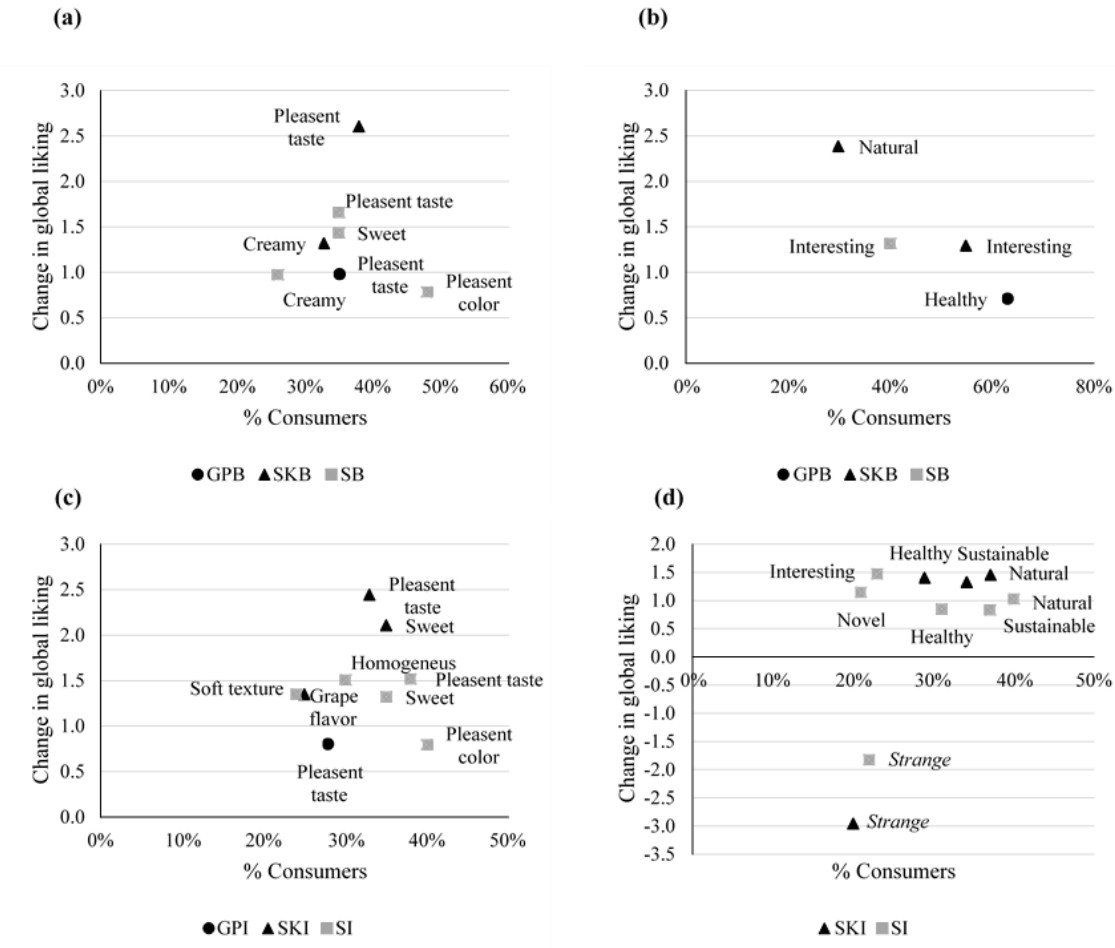

Figure 2. Results of the penalty analysis conducted for overall liking in blind (a, b) and informed (c, d) conditions. Sensory attributes are shown on the left (a, c), whereas non-sensory terms are shown on the right (b, d). Only significant attributes are shown. Attributes in regular font are "must have" attributes whereas "must not have" attributes are shown in italics. GPB: grape pomace blind; SB: seed blind; SKB: skin blind; GPI: grape pomace informed; SI, seed informed; SKI, skin informed.

Table 3. Cluster analysis based on the overall liking of the evaluated yogurts (grape pomace, seed, skin) in blind, expected and informed conditions.

	Blind			***Expected***			***Informed***		
	Cluster 1 (n = 26)	*Cluster 2 (n = 36)*	*Cluster 3 (n = 38)*	*Cluster 1 (n = 16)*	*Cluster 2 (n = 42)*	*Cluster 3 (n = 42)*	*Cluster 1 (n = 31)*	*Cluster 2 (n = 59)*	*Cluster 3 (n = 10)*
Grape pomace	6.5[a]	7.2[a]	6.7[a]	8.0[c]	5.7[a]	7.0[b]	5.9[a]	7.4[b]	7.6[b]
Seed	5.6[a]	7.8[b]	5.3[a]	4.9[a]	5.2[a]	7.6[b]	4.9[a]	7.4[b]	5.7[a]
Skin	7.4[b]	7.0[b]	3.9[a]	7.7[b]	6.1[a]	8.0[b]	6.4[b]	7.4[c]	1.8[a]

3.2. Expected testing

Results from the expected liking suggest that the different wine-making byproducts generated different hedonic expectations in consumers (Table 1). The expected liking generated by grape skin yogurt (7.1) was significantly higher than the grape pomace (6.6) and seed (6.2) yogurts ($p < 0.01$). Overall expected liking scores were above 6 in a 9-point hedonic scale, indicating that products were classified as "slightly liked". These results suggest that information regarding the origin of the byproducts did not affect the hedonic perception of the product in a negative way. In the description of the product we used the word "recovered" to inform consumers that the grape ingredients had been generated during the wine-making process. Other authors have evaluated the effect of the extrinsic labels used to refer to food byproducts on consumers' perception [22]. The authors highlighted that the words "upcycled" was the preferred label by consumers, although "recovered" was not studied.

The expected liking of the grape skin yogurt was the highest among all samples and evaluation conditions, suggesting that the hedonic perception of the concept of a yogurt containing grape skin extracts and dietary fiber was very favorable. The non-sensory terms highly associated with its description (selected by more than 70% of the consumers) were *healthy, antioxidant, high in fiber* and *novel* (Table 2). On the other hand, the grape seed yogurt was described as having a *woody flavor*, *astringent* and *heterogeneous texture*.

Three clusters with different expected liking patterns were identified using Ward's aggregation method (Table 3). Cluster 1 was composed by consumers which scored high expected liking values for the grape pomace and skin yogurts, with a lower expected likin for the seed yogurt. Consumers in clusters 2 and 3 scored all samples similarly. Consumers in cluster 3, which was a large cluster composed of 42 consumers, scored yogurts with values between 7.0 and 8.0, which suggest that the hedonic expectation of the products was very high. On the other hand, the 42 consumers in cluster 2 gave lower liking scores for the samples, within the range of 5.2 and 6.1. These results suggest that consumers within the largest clusters did not perceive significant differences between the samples. Rather, they showed consistently linear hedonic expectations of the samples as a whole, being higher in consumers in cluster 3.

3.3. Informed testing

Differences in informed liking scores between the different yogurt samples were also observed. The grape yogurt was significantly better liked ($p < 0.01$) than the grape seed

yogurt (Table 1). In general, informed liking achieved high liking scores (between 6.5 and 7.0).

The grape skin yogurt was significantly different from the other yogurt samples in terms of its color (*pleasant color*), taste (perceived with a higher *grape flavor*, less *milky* and described with a *disgusting taste*) and texture (increased frequency of *firm*) (Table 2). The grape seed yogurt was described with a woody flavor, and its texture was considered less *firm*, *soft* and *homogeneous*, and *grainier*. In general, yogurts containing dietary fiber and wine-making byproducts were described as *healthy, antioxidant, sustainable, high in fiber, natural, novel* and *interesting*. Among samples, the grape skin yogurt was perceived as more *antioxidant* and less *familiar*.

The penalty analysis showed that *pleasant taste* was a "must have" attribute in the grape pomace yogurt, which was selected in the ideal but missed in the yogurt sample by 28% of the consumers. In the grape skin yogurt, *pleasant taste, sweet* and *grape flavor* were identified as "must have" attributes which affected overall liking between 1.3 and 2.4 points. There results show a clear direction for reformulation towards the improvement of the yogurt's taste. In the case of the grape seed yogurt, overall liking was affected by missing the "must have" attributes *pleasant color, pleasant taste, sweet, soft texture* and *homogeneous texture*.

The informed evaluation condition gave place to "must not have" non-sensory attributes. This was the case of the grape skin and seed yogurts, where strange penalized overall liking by 2.9 and 1.8, respectively. *Healthy, sustainable* and *natural* were "must have" non-sensory attributes in grape skin and seed yogurts. This indicates that more efforts are needed to promote the health and sustainability message of the yogurts to improve their overall liking. In addition, *novel* and *interesting* were also "must have" attributes in the grape seed yogurt.

Finally, three clusters were also identified under informed conditions, with a predominant cluster with 59 consumers (cluster 2), and two smaller clusters (31 and 10 consumers in cluster 1 and cluster 3, respectively) (Table 3). Cluster 2 showed that all consumers scored the different yogurt samples similarly with a high liking (7.4), suggesting that possibly the hedonic expectations of the yogurts had been met. These results are promising as most consumers were in this cluster (n = 59). Cluster 1 showed consumers with a higher liking score for the grape skin yogurt. In cluster 3, which was represented only by 10 consumers, there was a clear preference for the grape pomace yogurt and a clear dislike for the grape skin yogurt.

3.4. Differences between conditions

There was a significant effect of the evaluation condition on consumers' liking scores ($F = 6.2, p < 0.01$), indicating that expectations were not fulfilled. The interaction yogurt type * evaluation condition was also significant ($F = 12.2, p < 0.001$), suggesting that the degree in which the expectations were met depended on the type of byproduct used as ingredient (grape pomace, grape seed or grape skin).

In the grape skin yogurt, the *pleasant color* attribute was highly selected by consumers in all the evaluation conditions, suggesting that color innovations in a purple palette for yogurts could be marketable. The influence of color on food preference has been extensively studied. Food coloring can influence flavor identification, perception and preferences, and can even dominate other flavor sources of information, such as labelling and taste [34]. In blind conditions the grape skin yogurt was significantly more cited as *pleasant color,* which may have influenced in the greater selection of the terms *grape flavor* and *antioxidant*. Therefore, the incorporation of a purple palette may be a potential marketing strategy in the development of yogurts containing grape ingredients to promote the perception of its antioxidant properties.

The grape skin yogurt created significantly high expectations that were not met in the informed conditions, which may be due to a lower grape flavor than expected. This is in line with the product reformulation direction identified in the penalty analysis, which showed that it is necessary to increase the *grape flavor, sweet* and *pleasant taste* attributes.

In general, the effect of information on the origin of the wine-making byproduct ingredients and the incorporation of dietary fiber significantly increased ($p < 0.001$) the frequency of selection of the attributes *antioxidant, high in fiber, satiety, sustainable* and *novel* compared to blind conditions. These associations, together with the overall liking scores which were similar or higher in the informed conditions compared to the blind test, suggest that the combination of winery byproduct extracts and dietary fiber may be used to target a market niche composed by environmentally and health conscious consumers. This is in agreement with Bhatt et al., (2017) who described that value-added surplus products were perceived as a different food category from conventional and organic foods.

4. Conclusions

The present study showed that overall liking was influenced by the type of byproduct, the evaluation condition and its interaction. Liking in expected and informed conditions

was significantly higher than in blind conditions, suggesting that information regarding the origin of the ingredients improved the overall liking of the yogurts. The improved hedonic perception of the yogurts in informed conditions may be explained by the positive associations with non-sensory terms such as *healthy, antioxidant, high in fiber, satiety, sustainable* and *novel*. The penalty analysis showed a clear product reformulation direction towards the improvement of the skin and seed yogurts taste. In addition, the overall liking of seed and skin yogurts was penalized when they were not considered healthy, natural and sustainable. Therefore, other product extrinsic cues should be used, such as packaging, to increase the perceived healthiness, sustainability and natural image of the products.

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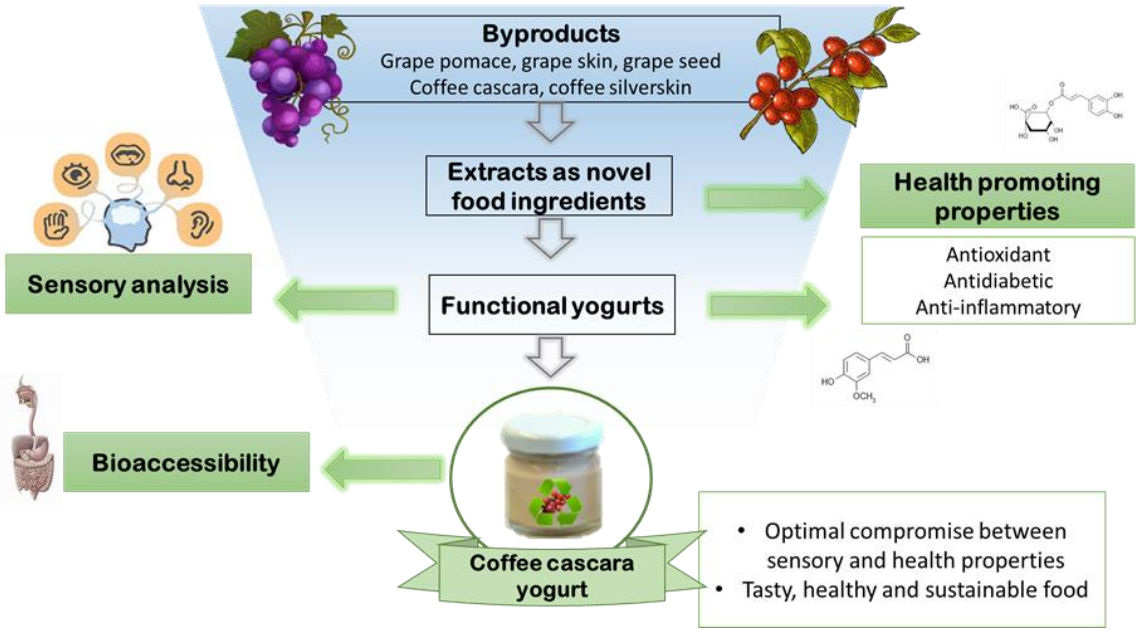
CHAPTER 3

Comparative study of the sensory quality and health promoting properties of yogurts containing coffee and wine-making byproduct extracts

This chapter provides a comparative evaluation of the yogurts formulated with coffee and wine-making byproducts. Results of the present study were submitted to the following journal for revision:

Iriondo-DeHond, M., Iriondo-DeHond, A., Herrera, T., Miguel, E. & del Castillo, M.D., 2019. Comparative study of the sensory quality and health promoting properties of yogurts containing coffee and wine-making byproduct extracts



Comparative study of the sensory quality and health promoting properties of yogurts containing coffee and wine-making byproduct extracts

Maite Iriondo-DeHond [1,2], Amaia Iriondo-DeHond [2], Teresa Herrera [2], Eugenio Miguel [1], María Dolores del Castillo [2]*

1 Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), N-II km 38,200, 28800 Alcalá de Henares, Spain.

2 Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), C/ Nicolás Cabrera, 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

Abstract

This study compared yogurts containing coffee and wine-making byproduct extracts as novel ingredients. For this purpose, the sensory acceptance, chemical profile and health-promoting properties of different yogurts were analyzed. Coffee (cascara and silverskin) and wine-making (grape pomace, seed and skin) extracts showed antioxidant (ORAC, ABTS, DPPH and intracellular ROS), antidiabetic (α-glucosidase inhibition) and anti-inflammatory (NO determination) properties. These properties were attributed to the presence of chlorogenic acid, ferulic acid and caffeine in the coffee byproducts and cyanidin-3-O-glucoside chloride and catechin in wine-making byproducts (detected by CZE). Among yogurt formulations, coffee cascara yogurt showed a high sensory acceptance (6.96), high antioxidant capacity (5.03 ± 0.11 µTE/g yogurt), the best inhibition of α-glucosidase activity (83%) and a significant ($p < 0.05$) reduction of NO levels at 10 mg/mL. Antioxidant and antidiabetic properties of cascara yogurt were also observed after *in vitro* digestion. In conclusion, a healthy and sustainable yogurt containing coffee cascara extract with antioxidant and antidiabetic properties after *in vitro* digestion was developed.

Keywords: coffee and wine-making byproducts; dietary fiber; functional food, novel ingredient; phytochemicals; yogurt

1. Introduction

Coffee and wine are two of the most consumed beverages all over the world. In 2016, more than 9 and 77 million metric tons of green coffee beans and grapes were produced, respectively [1]. The conversion of the coffee and grape berries into the popular beverages is responsible for the generation of a large amount of byproducts which represent an environmental issue. Approximately 90% of the edible parts of the coffee berry are discarded during its conversion into the coffee beverage [2], whereas wine-making byproducts account for about 20-30% of the weight of the grape [3]. Therefore, the valorization of byproducts from the coffee and wine industries is essential to promote the development of a more sustainable food system.

Coffee cascara, also known as coffee pulp [4–6], corresponds to the skin and pulp fraction generated in the wet processing during the obtention of the green coffee beans. Coffee silverskin is the tegument enclosing the coffee bean and is the only byproduct generated during the roasting process. The nutritional profile of coffee cascara and silverskin includes large amounts of carbohydrates, proteins, fat and minerals, and are considered good sources of dietary fiber [7]. In addition, several bioactive compounds with potentially health-promoting properties have been described in both coffee cascara and silverskin byproducts, including hydroxycynnamic acids (chlorogenic acids, ferulic acid), flavan-3-ols (epicatechin, catechin), flavonols (rutin), anthocyanins (cyanidin-3-rutinoside) and tannins [5,8–10].

Grape pomace containing grape seeds, pulp and skins is the main residue derived from the wine-making activity. The principal components of grape pomace are moisture, soluble sugars, peptic substances, cellulose and lignin [11]. Biologically active compounds recovered from wine-making by-products include hydroxybenzoic acids (p-hydroxybenzoic acid, protocatechuic acid, tannic acid, vanillic acid, gallic acid derivatives and syringic acid), flavanols (catequin), flavonols, anthocyanins, and stilbenes (resveratrol) [12,13].

Over the last years, innovative approaches have focused on valorizing food byproducts as food ingredients due to the extensive information available on the health-promoting activity related to their content on bioactive compounds. Whereas wine-making byproducts have been widely used in food formulations [14], limited progress has been achieved in the use of coffee byproducts as ingredients in functional foods. Moreover, there is a lack of knowledge on the antidiabetic properties of coffee and wine-making byproducts when present in complex food matrices, such as yogurts. This way, the aim of the present study was to compare coffee and wine-making byproducts for their use as

functional ingredients and to identify the best candidate for the development of sustainable and health-promoting yogurts focusing on their biological and sensory properties. In this context, we a) analyzed the sensory acceptance, chemical profile and antioxidant, antidiabetic and anti-inflammatory properties of the byproduct extracts and formulated yogurts, and b) studied the bioaccessibility after *in vitro* digestion of the yogurt formulation which presented the best properties. To our knowledge, this is the first time that coffee cascara is used in yogurt for the vehiculization of its bioactive compounds.

2. Materials and Methods

2.1. Extracts

2.1.1. Laboratory prepared extracts

Coffee cascara and silverskin from Arabica species and Colombian origin were kindly provided by Supracafé S.A. (Móstoles, Spain). Coffee cascara was generated during the obtention of the green coffee beans processed with the wet method. The coffee cascara used in this study was composed of the outer skin of the coffee berry and pulp. Coffee cascara was subjected to a sanitation process involving the use of a carbon dioxide atmosphere (MABA-PEX process). Coffee cascara was certified as safe for consumptions by Coffee Consulting S.L. as no chemical nor biological contaminants were detected in samples. Coffee beans were roasted using a PROBAT roaster (Emmerich am Rhein, Germany) for 13 minutes at 220 ºC and coffee silverskin was generated during this process.

Aqueous extracts were produced as described in the patent [15]. Briefly, 50 g of coffee cascara or silverskin were added per water liter. These mixtures were stirred for 10 minutes at 100 ºC, filtered and the filtrates were freeze-dried.

2.1.2. Commercial extracts

Commercial whole grape pomace, grape seed and grape skin hydroalcoholic extracts were purchased from Natac (Madrid, Spain).

2.2. Food samples

2.2.1. Yogurt samples

Six set-type yogurt formulations were prepared: coffee cascara, coffee silverskin, grape pomace, grape skin, grape seed yogurts and a control yogurt without byproduct extract. Yogurt samples were made using whole UHT cow milk (3.6% fat, 3% protein, 4.8%

sugar), starter culture YO-MIX 300 (Danisco), 3.1% (w/v) skimmed milk powder and 6% (w/v) inulin (Orafti®GR, Beneo). Inulin was added as a fiber for technological and nutritional reasons, to improve texture properties and to increase dietary fiber content in order to reduce the glycemic index of yogurts, respectively. Byproduct extracts were dissolved in water and added into the milk prior to fermentation at concentrations of 4 mg/mL (coffee silverskin), 5 mg/mL (wine-making byproducts) and 10 mg/mL (coffee cascara). These concentrations were determined by a preliminary pilot sensory acceptance test with 10 volunteers in which yogurts with different byproduct extract concentrations were analyzed. Coffee and wine-making byproduct extracts presented bioactive compounds (section 2.3) with antioxidant capacity determined by the assays described in section 2.4.1 (Table S1). Fermentation was completed when yogurts reached a pH of 4.5 and were stored at 4 ºC overnight. Yogurt manufacturing was performed in triplicate in three independent sessions to assure reproducibility of results.

Yogurt samples were prepared for further analysis by diluting each sample (10 g) in distilled water (2.5 mL), adjusting the pH to 4.0, incubating in a water bath for 10 min (45 ºC) and centrifuging to remove precipitated proteins. The supernatant was recovered and the pH was adjusted to 7.0 followed by another centrifugation to remove residual precipitated proteins. The supernatants were filtered with 0.45 µm nylon filters (Symta, Spain) and stored at -20 ºC.

2.2.2. Yogurt digests

The coffee cascara extract yogurt was selected due to its sensory, chemical and health-promoting properties to evaluate the bioaccessibility of its bioactive compounds after *in vitro* gastrointestinal digestion process. A yogurt without any extract was used as control. Cascara and control yogurts were digested in triplicate mimicking human digestion conditions as described by Holleebeeck et al.[16], and modified by Martinez-Saez et al. [17].

2.3. Chemical characterization of byproduct extracts and yogurts

2.3.1. Total phenolic content (TPC)

TPC analysis in extracts and yogurts was carried out by the Folin–Ciocalteu method [18]. A gallic acid calibration curve (0.01 – 1 mg/mL) was used for quantification and results were expressed as mg GAE/ g sample. Measurements were performed in triplicate.

2.3.2. Total anthocyanin content

Total anthocyanin content of the byproduct extracts and yogurts was estimated using the spectrophotometric pH differential method [19]. Results were expressed as mg cyanidin-3-O-glucoside chloride (C3G) equivalents/ g sample and all measurements were performed in triplicate.

2.3.3. Capillary zone electrophoresis (CZE)

Coffee cascara (10 mg/mL) and silverskin (4 mg/mL) extracts and wine-making byproduct extracts (5 mg/mL), yogurt formulations (1 g/mL) and digests (50 mg/mL) were subjected to capillary zone electrophoresis as described by del Castillo et al.[20] . Electropherograms (e-grams) were monitored at 280 nm, and spectra were collected from 190 to 600 nm. Bioactive compounds were detected and quantified by comparison of retention times and UV/VIS spectra using as references: gallic acid, caffeic acid, ferulic acid (FA), chlorogenic acid (CGA), rutin hydrate, catechin (CAT), C3G, procyanidin B2, genistein, glycitein, trigonelline hydrochloride, melatonin and caffeine (CAF) (Table S2). All analyses were performed in triplicate.

2.4. Health-promoting properties of byproduct extracts and yogurts

2.4.1. Antioxidant properties

The overall antioxidant capacity of extracts, yogurts and yogurt digests (control and cascara) was analyzed using the following methods:

- 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay:

DPPH free radical scavenging capacities of extracts and yogurts were evaluated according to Brand-Williams, Cuvelier and Berset (1995) adapted to microplate method. Trolox was used as a reference standard (0.25-2.5 mM in ethanol). All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- 2,2′-azinobis-(3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) assay

The trapping capacity of cationic free radicals was evaluated using the method of radical ABTS•+ bleaching described by Re et al. [22] and modified by Oki et al. [23] for its use in a microplate. Aqueous solutions of Trolox (0.15–2.0 mM) were used for calibration. All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay was applied according to the method of Ou et al.[24] as modified by Dávalos et al. [25]. All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- Physiological intracellular reactive oxygen species (ROS)

Normal rat small intestine epithelial cells (IEC-6) were kindly provided by the Bioanalytical Techniques Unit (BAT) of the Instituto de Investigación en Ciencias de la Alimentación (CIAL) (Madrid, Spain). Cells were cultured as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% v/v heat inactivated fetal calf serum (FBS), 50 U/mL penicillin and 50 µg/mL streptomycin and 1% v/v L-glutamine, at 37 ºC and in 5% CO2 in a humidified incubator (BINDER CB series 2010, Tuttlingen, Germany).

Prior to the study of physiological intracellular ROS, the effect of different concentrations of extracts, yogurts and yogurt digests (control and cascara) on cell viability was measured by the MTT assay [26] in order to select non-cytotoxic doses (Table S3 and S4). IEC-6 cells were treated with extracts in concentrations present in yogurts (coffee cascara extract (10, 100 and 1000 µg/mL), coffee silverskin extract (4, 40 and 400 µg/mL) and wine byproduct extracts (5, 50 and 500 mg/mL)), yogurts (1, 10 and 100 mg/mL) and yogurt digests (0.01, 0.1 and 1 mg/mL). DMSO (50%) was used as death control.

The determination of physiological intracellular ROS was performed following the same procedure used by Iriondo-DeHond et al., [27]. Tert-butyl hydroperoxide (tBOOH) 1 mM was used as a positive oxidation control and vitamin C as an antioxidant control. Then, a MTT assay was performed to normalize data by the number of cells per well. Non-treated control cells produced 100% of physiological ROS. Experiments were carried out in triplicate.

2.4.2. Antidiabetic properties

The α-glucosidase inhibition assay was carried out to study the antidiabetic properties of samples. The α-glucosidase inhibitory capacity of byproduct extracts, yogurts and digested yogurts (control and cascara), was analyzed following the methodology described by Berthelot & Delmotte[28] and Geddes & Taylor[29] and modified by Martinez-Saez et al.[30]. Results were expressed as percentage of α-glucosidase inhibition. All measurements were performed in triplicate.

2.4.3. Anti-inflammatory properties

Mouse macrophages (RAW 264.7) were cultured in the same conditions as intestinal cells. RAW 264.7 cells were treated with the same concentrations used for intestinal cells and only non-cytotoxic concentrations were used for the analysis of anti-inflammatory properties (Table S3 and S4). Anti-inflammatory properties were studied by measuring nitric oxide (NO) production in RAW 264.7 cells by inducing inflammation with E. coli lipopolysaccharide (LPS)[31]. Experiments were carried out in triplicate.

2.5. Consumer acceptance analysis

Consumers (n = 70) were recruited at the Instituto de Investigación en Ciencias de la Alimentación (CIAL) (Madrid, Spain). The test consisted of a hedonic evaluation of the coffee and wine-making byproduct yogurts and the control yogurt. Yogurt samples (30 mL) were served at 7 ºC using individual plastic containers identified with a random 3-digit code. Samples were given in blind conditions and were served in completely randomized order. Consumers were asked to rate the acceptance on appearance, smell, flavor, texture and overall acceptance of the samples by using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely).

2.6. Statistical analysis

A one-way analysis of variance (ANOVA) followed by Tukey's test for mean comparisons was used to highlight significant differences among samples. All statistical analyses were performed using IBM SPSS Statistics 24.

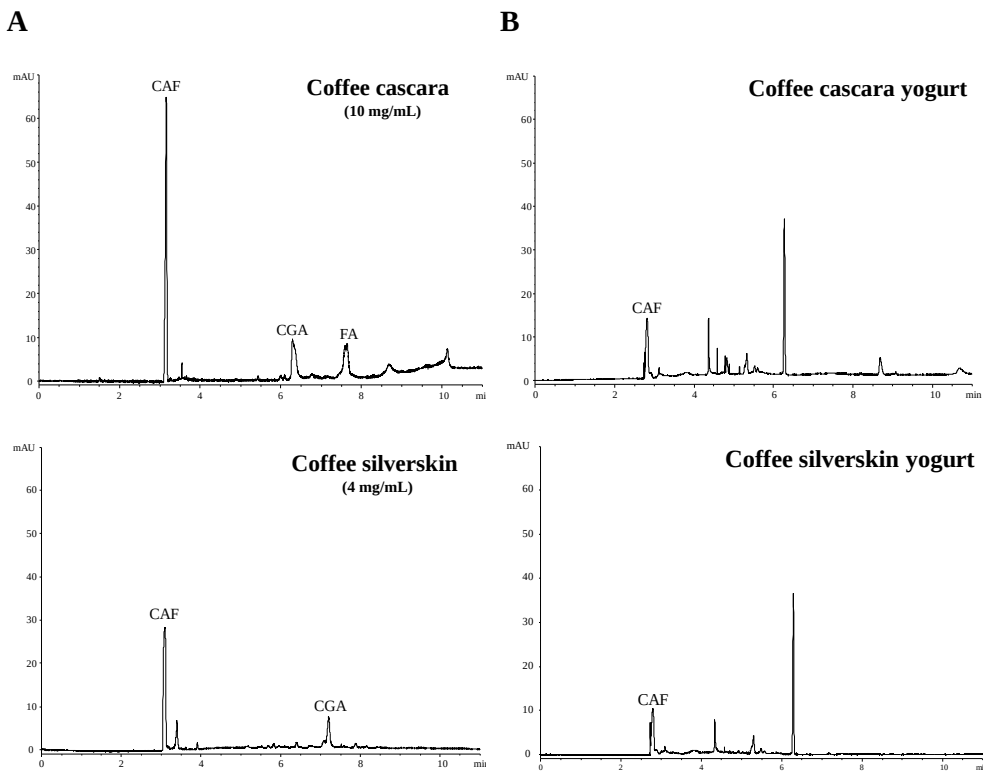
3. Results

3.1. Chemical characterization of yogurts

Yogurts containing grape pomace, skin and seed extracts presented TPC of 0.1 ± 0.01 mg GAE/g yogurt, 0.9 ± 0.03 mg GAE/g yogurt and 0.9 ± 0.02 mg GAE/g yogurt; respectively. The lowest TPC was observed in coffee silverskin yogurt (0.05 ± 0.00 mg GAE/g yogurt), whereas coffee cascara yogurt showed a higher value (0.13 ± 0.02 mg GAE/g yogurt). TPC differences were not significant between samples ($p > 0.05$). Coffee silverskin extract also presented the lowest value of TPC (37.94 ± 0.57 mg GAE/g extract) among the studied extracts (Table S1). Anthocyanins were just found in the grape skin yogurt (0.04 ± 0.01 mg C3G eq./g yogurt). Grape pomace and skin were the only extracts were anthocyanins were detected (Table S1).

Bioactive compounds present in yogurts and extracts were also identified by CZE (Figure 1). CAF was identified in yogurts made with coffee byproduct extracts (cascara

and silverskin). The e-gram of the coffee cascara extract showed three peaks with a spectrum matching CAF, CGA and FA, migrating at 2.9 min, 6.4 min and 7.1 min, respectively. In the coffee silverskin extract, CAF and CGA were also detected. CAF was the major component in both extracts. Considering phytochemical content per gram of extract, CAF content was significantly higher ($p < 0.001$) in coffee silverskin extract (34.87 ± 1.85 mg CAF/g extract) compared to the coffee cascara extract (17.42 ± 3.28 mg CAF/g extract). The content of CGA was also significantly higher ($p < 0.001$) in coffee silverskin extract (11.50 ± 0.98 mg/ g extract) than in coffee cascara extract (6.90 ± 0.41 mg/ g extract). FA was detected in coffee cascara extract (12.99 ± 0.17 mg/ g extract) but it was not detected in coffee silverskin extract at the concentration of 4 mg/mL used in the CZE analysis.



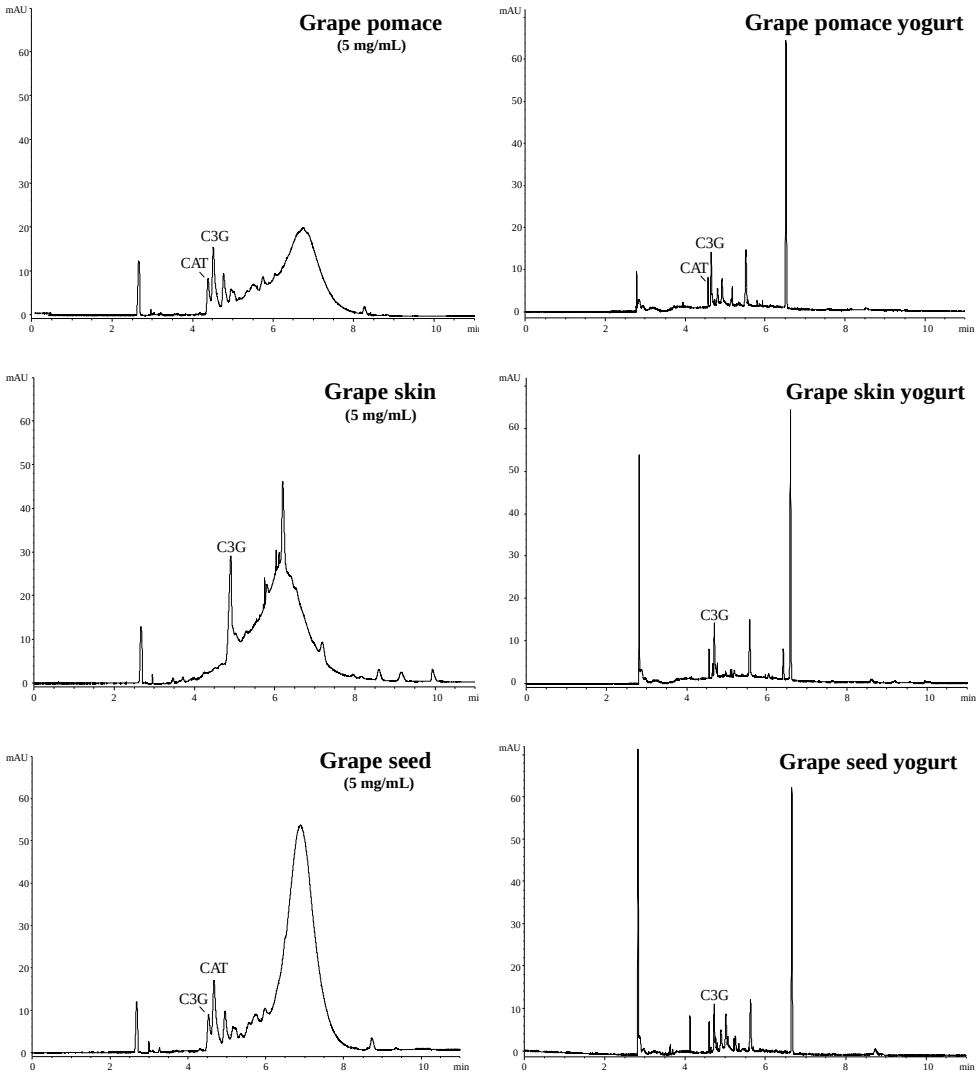


Figure 1. E-grams recorded at 280 nm showing the identified compounds from coffee and wine-making byproduct extracts (at the concentrations used in the corresponding yogurts) and yogurts containing the extracts as food ingredients (1 g/mL). Peak identification: CAF, caffeine; CGA, chlorogenic acid; FA, ferulic acid; CAT, catechin; C3G, cyanidin-3-O-glucoside chloride.

CAF content detected in coffee cascara and coffee silverskin yogurts was 110 and 60 µg CAF/g yogurt, respectively. Polyphenols were not detected. Anthocyanins were identified in yogurts containing wine-making byproduct extracts (grape, skin and seed). C3G was identified at 4.6 min in grape pomace, skin and seed extracts. CAT was identified at 4.7 min in grape pomace and grape seed extracts. The broad “hump”, migrating between 4.3 and 5.7 min, may be a derivative of anthocyanin compounds. In

wine-making byproduct extracts the highest content of C3G was found in grape skin extract (42.35 ± 10.31 mg C3G/g extract), followed by grape seed and grape pomace extracts (18.84 ± 1.35 and 15.68 ± 1.50 mg C3G/g extract, respectively) ($p < 0.005$). The content of C3G was similar (10 µg C3G/ g yogurt) among wine-making byproduct yogurts.

3.2. Health-promoting properties of yogurts

3.2.1. Antioxidant properties of yogurts

Results from the antioxidant capacity *in vitro* determinations in yogurts (Table 1), showed that the addition of 4 mg/mL of coffee silverskin extract did not significantly increase the yogurt's antioxidant capacity ($p > 0.05$). Yogurts containing coffee cascara, grape pomace, skin and seed extracts showed a similar significant increase in their antioxidant capacity compared to the control yogurt ($p < 0.01$), although grape skin yogurt showed a significant lower value in DPPH radical scavenging than the grape

The assessment of the *in vitro* overall antioxidant capacity evaluated by ORAC, ABTS and DPPH methods showed that wine-making byproduct extracts were significantly ($p < 0.001$) better antioxidants than coffee byproduct extracts (Table S1).

With regard to the study of intracellular ROS formation (Table 2), the correct physiological redox status of cells was confirmed since the oxidation control (t-BOOH 1 mM) significantly increased ($p < 0.05$) ROS and the antioxidant standard (vitamin C 10 µg/mL) significantly reduced ($p < 0.05$) ROS levels. No significant effects ($p > 0.05$) were observed when cells were treated with yogurts (1, 10 and 100 mg/mL) compared to control non-treated cells (Table 2). However, coffee and wine-making byproduct extracts significantly reduced ($p < 0.05$) physiological intracellular ROS. The decrease in physiological intracellular ROS levels was similar ($p > 0.05$) to that observed for vitamin C (10 µg/mL, 32.40 ± 12.01 % ROS).

3.2.2. Antidiabetic properties of functional yogurts

Although all coffee and wine-making byproduct extracts inhibited the activity α-glucosidase (Table S1), only yogurts containing coffee byproduct extracts and grape skin extract significantly inhibited the activity of the enzyme ($p < 0.01$) compared to the control (Table 1). Coffee cascara yogurt showed the highest inhibition of the activity of α-glucosidase (83%), which was significantly higher than the rest of the yogurt formulations ($p < 0.01$).

Table 1. Antioxidant and antidiabetic properties of yogurts formulated with coffee and wine-making byproduct extracts.

		Coffee byproducts		*Wine-making byproducts*		
	Control	*Cascara*	*Silverskin*	*Grape pomace*	*Skin*	*Seed*
Antioxidant capacity						
ORAC (µmol TE/g yogurt)	1.79 ± 0.23[a]	5.03 ± 0.11[b]	2.82 ± 0.19[a]	6.15 ± 0.36[bc]	5.24 ± 1.07[bc]	7.26 ± 1.32[c]
ABTS (µmol TE/g yogurt)	0.42 ± 0.04[a]	3.26 ± 0.13[c]	1.01 ± 0.29[ab]	1.63 ± 0.05[b]	1.59 ± 0.36[b]	1.67 ± 0.38[b]
DPPH (µmol TE/g yogurt)	0.00 ± 0.00[a]	0.75 ± 0.17[c]	0.22 ± 0.07[ab]	0.73 ± 0.12[c]	0.47 ± 0.07[b]	0.90 ± 0.09[c]
*Antidiabetic properties**						
α-Glucosidase inhibition (%)	41.47 ± 5.72[a]	82.87 ± 2.14[c]	64.54 ± 4.05[b]	54.11 ± 3.96[ab]	63.81 ± 5.62[b]	53.10 ± 5.16[ab]

*Concentrations for antidiabetic analyses: α-glucosidase inhibition was conducted at 4 g/mL in yogurts containing coffee and wine-making byproducts.

Different letters denote statistically significant differences between formulated yogurts (Tukey test, $p < 0.05$).

Table 2. Effect of 24 h treatment with noted concentrations of extracts and yogurts on intracellular ROS generation in IEC-6 cells determined by the DCFH-DA probe. Oxidative damage was induced by t-BOOH (1 mM) and vitamin C (10 µg/ml) was used as an antioxidant control.

Extracts (µg/ml)	ROS (%)	Yogurts (mg/ml)	ROS (%)
Control	100.0 ± 0.0[d]	Control	100.0 ± 0.0[ab]
tBOOH (1 mM)	174.80 ± 34.25[e]	tBOOH (1 mM)	174.80 ± 34.25[c]
Vit C (10 µg/mL)	32.40 ± 12.01[ab]	Vit C (10 µg/mL)	32.40 ± 12.0[a]
		Control	
		1	123.10 ± 38.75[bc]
		10	97.49 ± 30.04[ab]
		100	83.44 ± 32.1[ab]
Coffee cascara		*Coffee cascara*	
10	45.66 ± 14.37[abc]	1	99.22 ± 33.29[ab]
100	35.96 ± 8.62[ab]	10	79.69 ± 27.69[ab]
1000	49.17 ± 24.79[abc]	100	87.37 ± 38.59[ab]
Coffee silverskin		*Coffee silverskin*	
4	65.74 ± 21.29[bcd]	1	101.21 ± 28.82[ab]
40	38.79 ± 11.66[abc]	10	87.80 ± 30.87[ab]
400	35.04 ± 9.72[ab]	100	85.69 ± 37.39[ab]
Grape pomace		*Grape pomace*	
5	40.26 ± 11.40[abc]	1	124.49 ± 41.84[bc]
50	53.06 ± 19.25[abc]	10	103.18 ± 37.28[abc]
500	82.27 ± 29.49[cd]	100	95.28 ± 37.33[ab]
Grape skin		*Grape skin*	
5	42.56 ± 12.65[abc]	1	108.02 ± 29.86[abc]
50	22.02 ± 6.66[ab]	10	97.98 ± 34.50[ab]
500	12.99 ± 4.64[a]	100	96.29 ± 40.30[ab]
Grape seed		*Grape seed*	
5	41.50 ± 8.16[abc]	1	119.72 ± 32.41[abc]
50	59.76 ± 17.36[bcd]	10	83.15 ± 29.42[ab]
500	63.77 ± 24.53[bcd]	100	79.14 ± 35.9 [ab]

Data represent means ± SD of 3 independent experiments. Different letters denote statistically significant differences between extracts or yogurts (Tuckey test, $p < 0.05$).

3.2.3. Anti-inflammatory properties of functional yogurts

Yogurts containing coffee cascara and coffee silverskin at 10 mg/mL significantly reduced ($p < 0.05$) levels of induced NO (Figure 2B). This effect is also observed when cells were treated with pure extracts (Figure 2A). No significant changes ($p > 0.05$) in NO levels were observed in macrophages treated with yogurts containing wine byproduct extracts. However, grape pomace and grape seed extracts at 50 µg/mL significantly reduced ($p < 0.05$) the production of NO.

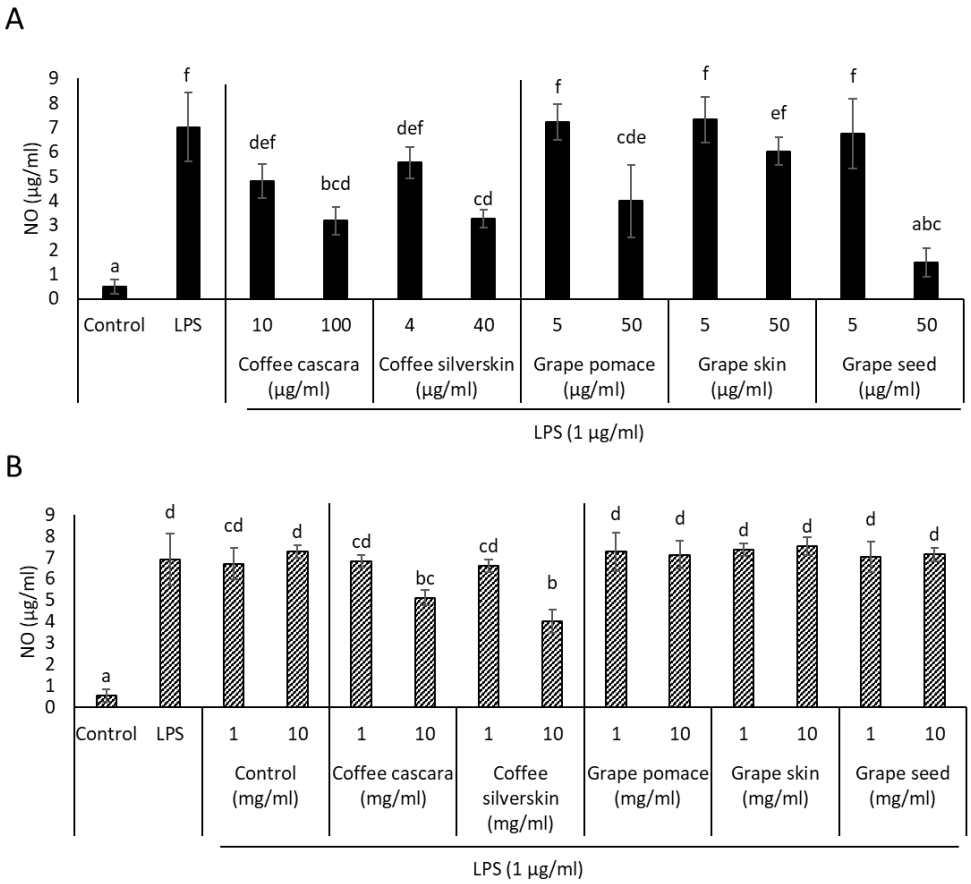


Figure 2. Anti-inflammatory effect of coffee and wine-making byproduct extracts (A) and yogurts containing the extracts as food ingredients (B) on nitric oxide (NO) generation in RAW264.7 induced by LPS (1 µg/ml). Cells were pre-treated with extracts or yogurts for 24 h. Then, macrophages were treated with samples and LPS for 24 h. Supernatants from treated cells and NO standards were mixed with Griess reagent (1:1) and absorbance was measured at 550 nm. Data represent means ± SD of 3 independent experiments. Different letters denote statistically significant differences between all treatments (Tukey test, $p < 0.05$).

3.3. Consumer analysis of yogurts

Results from the hedonic test with untrained consumers (n = 70) are shown in Table 3. The basal formulation (control yogurt) showed high scores in all attributes (mean values > 6.8). The grape seed yogurt showed a significantly low score for the smell attribute ($p < 0.01$). Coffee silverskin yogurt showed significantly lower taste scores ($p < 0.05$) than the rest of the formulated yogurts. Coffee cascara, grape seed and grape pomace showed similar acceptance scores for taste than the control ($p > 0.05$). All yogurt formulations had similar texture properties ($p > 0.05$). Overall, yogurts containing coffee cascara and wine-making byproduct extracts showed high acceptance values (6.22 – 6.96), which were similar to the control (Figure 3). Only the coffee silverskin yogurt showed significantly lower overall acceptance than the rest of the yogurt formulations ($p < 0.01$). The cascara yogurt was the only formulation that obtained similar acceptance scores than the control for all the individual attributes evaluated ($p > 0.05$).

Table 3. Mean consumer acceptance scores on appearance, smell, taste and texture of control and yogurts formulated with coffee and wine-making byproducts using a 9 point hedonic scale.

	Appearance	Smell	Taste	Texture
Control	7.69 ± 1.26^{c}	6.82 ± 1.71^{b}	7.38 ± 1.44^{c}	7.67 ± 1.26^{a}
Coffee cascara	6.95 ± 1.83^{abc}	6.87 ± 1.49^{b}	6.82 ± 1.55^{bc}	6.82 ± 1.47^{a}
Coffee silverskin	6.59 ± 1.60^{ab}	6.49 ± 1.62^{ab}	4.36 ± 2.08^{a}	6.72 ± 1.81^{a}
Grape seed	5.93 ± 1.49^{a}	5.44 ± 1.25^{a}	6.48 ± 1.63^{bc}	6.74 ± 1.43^{a}
Grape skin	7.30 ± 1.26^{bc}	5.78 ± 1.40^{ab}	5.89 ± 1.99^{b}	6.85 ± 1.35^{a}
Grape pomace	5.96 ± 1.34^{a}	5.96 ± 1.31^{ab}	6.52 ± 1.60^{bc}	7.04 ± 1.43^{a}

Data represent means ± SD. Different letters in columns denote statistically significant differences between yogurts (Tukey test, $p < 0.05$).

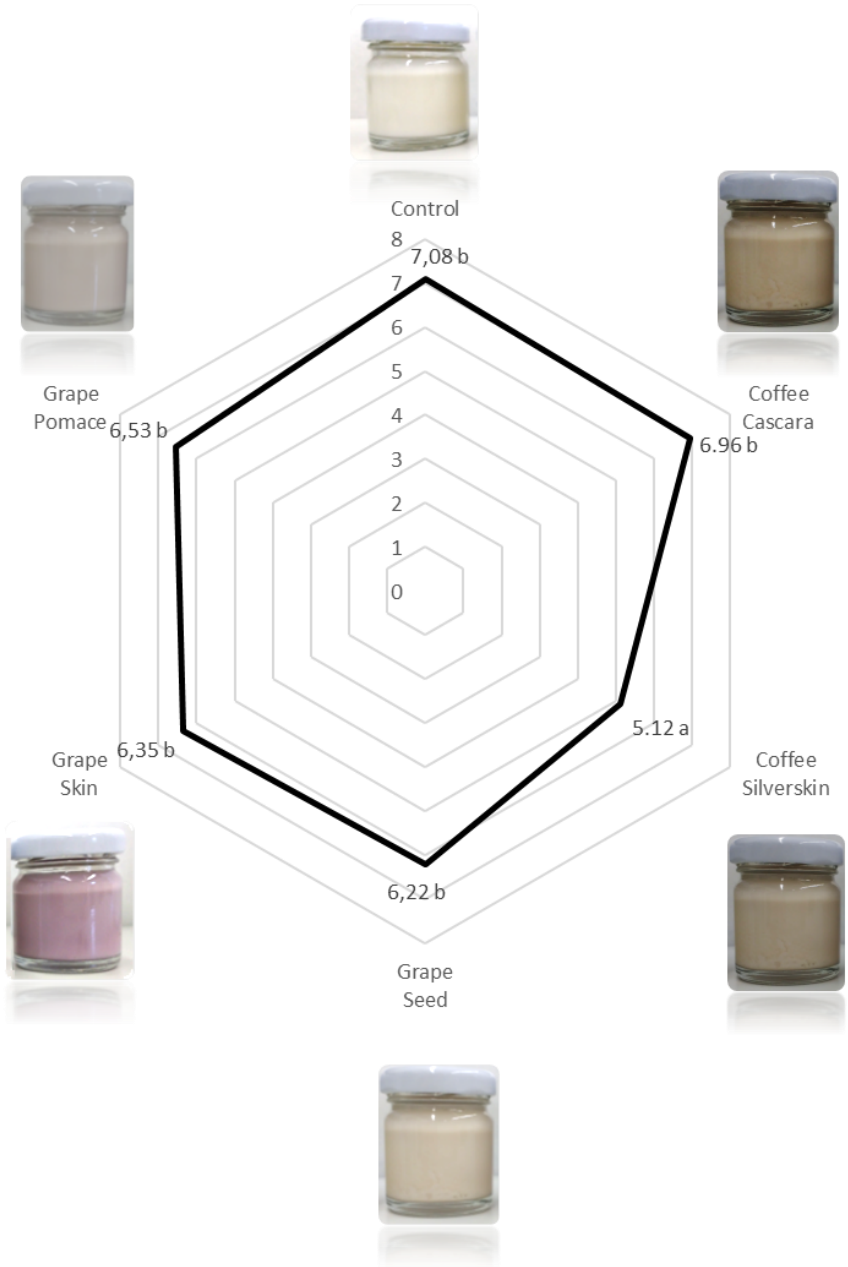


Figure 3. Consumer (n = 70) overall acceptance of yogurts formulated with coffee byproducts (cascara and silverskin) and wine-making byproduts (grape pomace, skin and seed) measured in a 9 point hedonic scale. Different letters denote statistically significant differences between yogurts (Tukey test, $p < 0.01$).

3.4. Bioaccessibility of bioactive compounds in coffee cascara yogurt

3.4.1. Chemical characterization of digested cascara yogurt

The TPC of the digested cascara yogurt was 4.90 ± 0.13 mg GAE/ g digest, which was similar to the control yogurt 4.83 ± 0.64 mg GAE/ g digest. E-grams of digested coffee cascara and control yogurts (Figure 4) did not show the presence of any of the standard compounds detected in the extracts used as ingredients in yogurts.

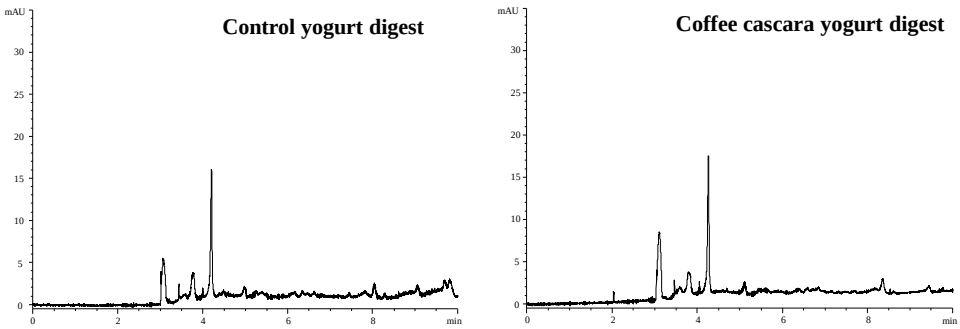


Figure 4. E-grams recorded at 280 nm from the digests of the control yogurt and yogurt containing coffee cascara extract (50 mg/mL).

3.4.2. Health-promoting properties of yogurts after *in vitro* digestion

3.4.2.1. Antioxidant properties of digested cascara yogurt

The antioxidant capacity of digested control and coffee cascara yogurts analyzed with the ABTS radical was similar (186.43 ± 9.71 µmol TE/g digest, 187.04 ± 17.69 µmol TE/g digest; respectively). However, the antioxidant capacity measured with the ORAC method was significantly higher in digested cascara yogurt (100.34 ± 12.77 µmol TE/g digest) than the control (76.04 ± 10.94 µmol TE/g digest) ($p < 0.05$).

Considering the effect of yogurt digests on intracellular ROS levels, concentrations of 0.01 and 0.1 mg/mL were tested on intestinal cells (Table S4). Control yogurt digests did not alter the antioxidant status of IEC-6 cells compared to non-treated control cells. However, a dose of 0.01 mg/mL of the cascara yogurt digest significantly reduced ($p < 0.05$) physiological intracellular ROS levels to 75 ± 10 %.

3.4.2.2. Antidiabetic properties of digested cascara yogurt

The antidiabetic properties of the cascara yogurt remained after the *in vitro* digestion process. Coffee cascara yogurt significantly ($p < 0.001$) inhibited the activity of the α-glucosidase enzyme (20.06 ± 2.81 %) compared to the control yogurt (7.79 ± 2.16 %).

3.4.2.3. Anti-inflammatory properties of digested cascara yogurt

The two lower concentrations of yogurts (1 and 10 mg/mL) and all the concentrations tested for digests were used for NO determination. However, none of the tested concentrations was able to reduce NO levels induced by LPS.

4. Discussion

The aim of this study was to compare yogurts containing coffee and wine-making byproducts as functional ingredients and to identify the best one focusing on their biological and sensory properties. With regard to the composition of yogurts, the quantity of bioactive compounds depended on the type and dose of byproduct extract added into the yogurt matrix. Polyphenols accounted for approximately 4% and 8% of the total composition of coffee silverskin and cascara extracts, and for 25%, 32% and 39% in the grape pomace, skin and seed extracts, respectively. A pilot sensory test allowed to determine the concentration in which the different byproducts could be added into the yogurt. Although wine-making byproduct extracts had higher TPC than coffee byproducts, the addition of 5 mg/mL of the wine-making byproduct extracts resulted in a similar TPC (0.09 mg GAE/g yogurt) than adding 10 mg/mL of the coffee cascara extract (0.13 mg GAE/g yogurt).

CZE was used to identify which phytochemicals could be contributing to the health-promoting properties of the byproducts extracts, as the presence of phenolic compounds in grapes and coffee has been previously correlated with antioxidant, antidiabetic and anti-inflammatory characteristics [32–34]. CGA and FA were not detected in coffee cascara and silverskin extract yogurts, although the theoretically expected contents of the bioactive compounds (70 µg CGA/g and 50 µg CGA/g for cascara and silverskin extracts, respectively; and 130 µg FA/g cascara extract) were over the detection and quantification limits of the CZE method. CAF was detected in coffee cascara and silverskin yogurts, although the observed contents were 35% and 58% lower than the theoretically expected, respectively. A portion of 125 g of coffee cascara and silverskin yogurts would have 13.75 mg and 11.25 mg of CAF, which is far below the European safety threshold of 400 mg per day for the healthy adult population and 200 mg per day for pregnant woman and children [35]. In comparison to some of the most popular caffeinated beverages, CAF content of the formulated yogurts was lower than a 250 mL

cup of coffee (66.88 mg of CAF), a 33 cl can of cola soda (31.05 mg of CAF) or a 250 mL cup of green tea (30.13 mg of CAF) [36]. In relation to yogurts containing wine-making byproducts, C3G was detected in grape pomace, skin and seed yogurts, although the observed contents were approximately 95% lower than the theoretically expected.

This decrease between the expected and observed content of bioactive compounds of yogurts containing coffee and wine-making byproduct extracts could be due to several factors, such as compound degradation during milk fermentation or a matrix effect which could be interfering with CZE detection. There is limited information on the metabolic activity of yogurt starter culture bacteria on phenolic compounds. Some Lactobacillus species, such as L. plantarum, have been described to metabolize plant-derived phenolic compounds, such as hydroxycinnamic acids and tannins [37]. Therefore, further analyses are needed to study whether the starter culture could have modified the initial phenolic profile of the byproduct extracts into other derivatives during milk fermentation. On the other hand, there is evidence of a matrix effect concerning protein-polyphenol interactions between milk proteins and phytochemicals. An immediate decrease of polyphenol compounds after 24 h of adding a strawberry preparation to milk for yogurt elaboration, observing a decrease within a range of 29% to 60% in individual polyphenols [38]. Interestingly, polyphenol availability increased after 28 days of storage, probably due to the linkage break between proteins and polyphenols and their consequent release.

Regardless of the lower TPC observed in yogurt samples, the addition of coffee and wine-making byproduct extracts to yogurt provided an increase in its antioxidant capacity compared to the control, except for the coffee silverskin yogurt. Results indicated that higher concentrations of coffee silverskin extract are needed to improve the overall antioxidant capacity of the yogurts, which would only be possible if other ingredients are used to mask the poorly accepted taste of the extract. Yogurt containing coffee cascara extract showed antioxidant values in the same range as yogurts containing wine-making byproduct extracts. The overall observed antioxidant capacity of coffee silverskin and cascara yogurts was 59% and 68% lower than the antioxidant capacity theoretically expected (considering the sum of the contribution from the bioactive peptides in the control yogurt and the coffee byproduct extracts). This difference was even more notable in yogurts containing wine-making byproducts, which was 90% lower than the theoretically expected grape pomace, skin and seed yogurts. This reduction in the antioxidant capacity has been previously associated to strong protein-polyphenol interactions in other dairy foods fortified with phenol compounds, such as strawberry yogurt formulations, tea beverage with milk or milk with chocolate [39–41].

In this study, coffee and wine-making byproduct extracts reduced 18% to 88% intracellular ROS levels in rat intestinal cells. To the best of our knowledge, this is the first time the antioxidant capacity of coffee cascara and silverskin extracts is studied in intestinal cells. The antioxidant effects in intracellular ROS in yogurts containing coffee and winemaking byproducts were not observed. This could be due to the matrix effect that yogurt compounds exert on extracts, hiding their functional groups.

Intestinal α-glucosidases are the key enzymes responsible for carbohydrate digestion and absorption. Their inhibition has been proven effective in both preventing and treating diabetes by targeting postprandial hyperglycemia [33]. The antidiabetic properties of yogurts have been less studied. The inhibition of α-glucosidase activity has been measured in yogurts containing garlic, cinnamon and other herbs [42–44], but there is no previous reference of the effect of yogurt containing coffee or wine-making byproducts on the activity of diabetes key-enzymes. The greater addition of coffee cascara extract in yogurt led to the highest value of α-glucosidase inhibition (83%, $p < 0.01$). Curiously, the inhibition of the enzyme by the coffee silverskin extract in yogurt was similar to yogurts containing wine-making byproduct extracts ($p > 0.05$). This result was unexpected as the coffee silverskin extract had shown the lowest inhibition rate among byproduct extracts and was added into the yogurt at the lowest concentration (4 mg/mL). Additional research on the bioactive compound profile of the yogurts are needed to analyze whether this increase could be due to compound modifications during fermentation of coffee silverskin yogurts and to further understand the matrix effect, which could be different for each type of byproduct extract.

The antidiabetic effect of coffee silverskin extract has been previously associated with its *in vitro* capacity to inhibit the enzymatic activity of α-glucosidase [45]. However, to the best of our knowledge, the *in vitro* glucoregulatory properties of coffee cascara extract have been first described in this study. Our results showed that the α-glucosidase inhibition exerted by the coffee cascara extract was significantly higher (40.17 ± 0.51 %) than that of coffee silverskin extract (32.62 ± 1.41 %) ($p < 0.001$). Chemical characterization of these extracts by CZE showed significantly higher concentrations ($p < 0.001$) of CGA and CAF per gram of coffee silverskin extract (11.50 ± 0.98 mg CGA/g extract and 34.87 ± 0.38 mg CAF/g extract) than per gram of coffee cascara extract (6.90 ± 0.41 mg CGA/g extract and 17.42 ± 3.28 mg CAF/g extract). CGA exerts an inhibitory action against α-glucosidase activity [46]. Therefore, the detection and quantification of CGA in the coffee byproduct extracts could support the observed inhibition of the α-glucosidase activity. The higher α-glucosidase inhibition of the coffee cascara extract could be due to the presence of FA, which was only detected in this byproduct extract

(12.99 ± 0.17), and other bioactive compounds. FA has been identified as one of the main contributors to the antidiabetic properties of other food products such as corn bran, wheat aleurone and pear peels [47,48]. Structural analysis of FA has shown a high binding affinity for interaction with the α-glucosidase active site, which could explain its inhibitory effect [49]. The role of CAF in the inhibition of α-glucosidase is still unknown, although previous studies have suggested that caffeine intake was not substantially associated with risk of type 2 diabetes [50,51].

In relation to wine-making byproducts, the inhibition of the grape skin extract (76%) was higher than that of other commercial grape skin extracts (31% to 45%) [33]. More similar values were obtained when the same authors analyzed grape skin extracts from the Norton and Cabernet Franc varieties (85% and 70%, respectively). Grape seed extracts are potent inhibitors of α-glucosidase activity probably due to their content in catechin 3-gallates [52]. The seed extract showed the highest inhibition for α-glucosidase (80%) activity. Novel nutraceuticals with combined antioxidant and α-glucosidase inhibitory properties could be an effective therapy for managing postprandial hyperglycemia with minimal side effects [33,53–55]. This strategy is based on using a combination therapy for preventing diabetic complications, as chronic hyperglycemia causes oxidative stress [56], and oxidative stress plays a key role in the pathogenesis of vascular dysfunction in diabetes [57]. Our results suggest that coffee and wine-making byproduct extracts could exert this combined antioxidant and α-glucosidase inhibitory action. However, further in vivo studies are needed to evaluate their role as antidiabetic agents.

Regarding the anti-inflammatory properties, only yogurts containing coffee cascara and silverskin at 10 mg/mL reduced LPS-induced inflammation. Coffee cascara and silverskin at 100 and 40 µg/mL significantly reduced ($p < 0.05$) LPS-induced inflammation by 53% (Figure 2). To the best of our knowledge, there are no published studies regarding the analysis of coffee silverskin anti-inflammatory properties in cellular models. In contrast, there is only one recent study concerning the anti-inflammatory properties of coffee skin/pulp obtained from a semi-dry processing [58]. These authors observed that coffee pulp was capable of inhibiting the release of the pro-inflammatory cytokine IL-8 on gastric epithelial cells when inflammation was induced by TNF-α [59]. Both pure CAF and CGA showed anti-inflammatory properties by reducing NO levels in LPS-induced RAW 264.7 cells, the same model used in our study [60,61]. These results suggest that CAF and CGA could be the main contributors to the overall anti-inflammatory effects observed for coffee byproduct extracts.

No anti-inflammatory effects were observed for yogurts containing winemaking byproducts. However, grape pomace and seed extracts in concentrations of 50 µg/mL significantly reduced ($p < 0.05$) NO levels by 43% and 79%, respectively. The anti-inflammatory properties of grape pomace and seed extracts have been previously described in macrophages and in other cell lines [62–64]. The absence of anti-inflammatory properties in yogurts may be due to a possible matrix effect between winemaking byproducts and other components present in yogurts. Previous clinical studies also suggest the consumption of yogurts as a preventive strategy to reduce inflammation [65,66]. The anti-inflammatory effect of yogurts has been attributed to milk oligosaccharides and lactoferrin and to probiotics added to yogurts [65,66]. Therefore, it would be interesting to enhance the anti-inflammatory properties of the yogurt matrix by adding natural and sustainable extracts with anti-inflammatory, antioxidant and antidiabetic properties.

Our study is the first to analyze consumer acceptance of yogurts containing coffee silverskin and cascara byproduct extracts. In contrast, the addition of wine-making byproducts in yogurt has already been reported by several authors [67–70]. These studies showed that overall liking of the yogurts varied depending on the type of byproduct added, whether the byproduct was used as flour or as an extract, its dosage and its combination with other ingredients. Our results showed that all formulated yogurts achieved high overall acceptance scores besides the coffee silverskin yogurt, which had significantly lower ($p < 0.05$) overall liking and taste values compared to the rest of the yogurts. This indicates that coffee silverskin yogurt should be reformulated to improve its taste and overall acceptability. Yogurts containing wine-making byproducts achieved similar ($p > 0.05$) overall acceptance scores to the control, but significantly lower scores were observed in some individual attributes, such as appearance (grape pomace and seed yogurts, $p < 0.001$), smell (grape seed yogurt, $p < 0.01$) and taste (grape skin yogurt, $p < 0.05$). Coffee cascara yogurt was the only yogurt formulation that obtained a sensory profile for all its attributes similar to the control yogurt, which was highly accepted (Table 3).

Overall, results from this work showed that coffee cascara yogurt achieved the best sensory profile and the highest antioxidant, antidiabetic and anti-inflammatory properties. Therefore, coffee cascara extract was selected as the best candidate for its application as a food ingredient in yogurt. The antioxidant capacity of the control yogurt increased after *in vitro* digestion, probably due to further release of antioxidant peptides and amino acids encrypted in the milk proteins [71]. The antioxidant capacity of the coffee cascara yogurt (100.34 ± 12.77 µmol TE/g yogurt) was significantly higher ($p <$

0.05) than the antioxidant capacity of the control yogurt (76.04 ± 10.94 µmol TE/g yogurt) when measured by ORAC. Moreover, the antioxidant capacity of the coffee cascara yogurt increased by 93% after *in vitro* digestion. This increase in antioxidant capacity after *in vitro* digestion has also been observed in yogurts containing cinnamon, strawberry and peach polyphenols [38,72]. In peach and strawberry yogurts, the increased antioxidant capacity after digestion seemed to be a result from stomach acidification and enzymatic activity leading to the release of polyphenols from the protein/polysaccharide yogurt matrix. This suggested that despite the significant decrease in the TPC, the yogurt matrix allowed the protection of some compounds from degradation, becoming bioaccessible [38]. An increase in the inhibition of the enzyme α-glucosidase after *in vitro* digestion was not observed in the control and coffee cascara yogurts. However, the inhibition of the activity of α-glucosidase was still significantly higher ($p < 0.001$) in coffee cascara yogurt digests than in the digested control yogurt. Additional studies addressing the bioaccessibility and bioavailability of coffee cascara should be conducted as they are required for its authorization as a novel food category [73].

In conclusion, the present study showed the sensory acceptance, chemical profile and antioxidant, antidiabetic and anti-inflammatory properties of coffee and wine-making byproduct extracts for their application as ingredients in yogurt. We selected the coffee cascara extract as the optimum extract based on the mentioned parameters. Yogurts containing coffee cascara extract have the potential to be suitable functional foods, providing a novel dietary opportunity for diabetes management due to their combined antioxidant and inhibitory action against α-glucosidase. The results obtained in this research should be compared with additional in vivo studies to better understand the potentially health-promoting effects of coffee cascara.

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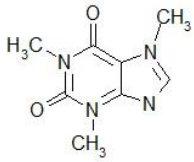
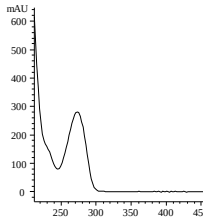
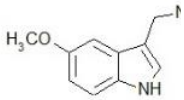
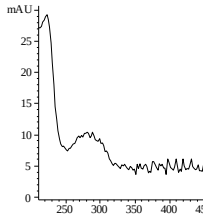
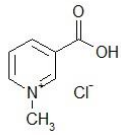
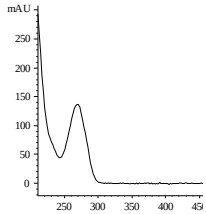
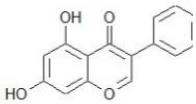
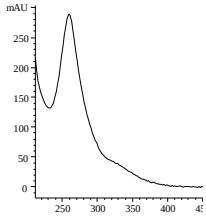
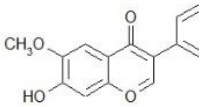
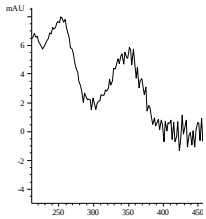
Table S1. Composition and *in vitro* bioactivity of coffee and wine-making byproduct extracts.

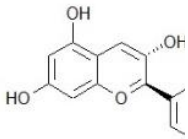
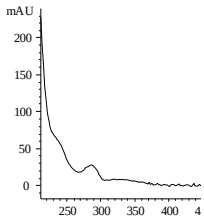
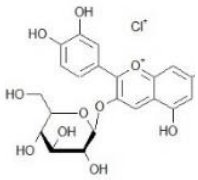
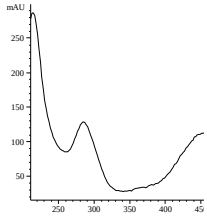
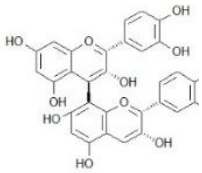
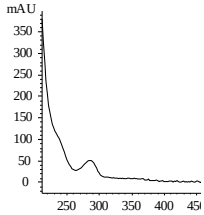
	Coffee byproducts		*Wine-making byproducts*		
	Cascara	*Silverskin*	*Grape pomace*	*Skin*	*Seed*
Chemical characterization					
TPC (mg GAE/g extract)	76.02 ± 0.77[b]	37.94 ± 0.57[a]	247.51 ± 13.65[c]	317.89 ± 15.47[d]	392.91 ± 7.84[e]
Anthocyanins (C3G eq./g extract)	n.d.	n.d.	2.22 ± 0.13[a]	85.11 ± 1.97[b]	n.d.
Antioxidant capacity					
ORAC (mmol TE/g extract)	1.68 ± 0.50[a]	1.55 ± 0.52[a]	5.97 ± 0.11[b]	6.21 ± 1.47[b]	7.12 ± 0.87[b]
ABTS (mmol TE/g extract)	0.80 ± 0.02[b]	0.40 ± 0.01[a]	4.50 ± 0.19[c]	6.25 ± 0.06[d]	7.75 ± 0.15[e]
DPPH (mmol TE/g extract)	0.25 ± 0.02[a]	0.15 ± 0.01[a]	2.77 ± 0.62[b]	3.09 ± 1.43[b]	3.74 ± 0.35[b]
*Antidiabetic properties**					
α-Glucosidase inhibition (%)	40.17 ± 0.51[b]	32.62 ± 1.41[a]	55.27 ± 1.82[c]	76.15 ± 1.43[d]	80.63 ± 0.26[e]

*Concentrations for antidiabetic analyses: α-glucosidase inhibition was conducted at 1 mg/mL in coffee and wine-making byproducts.

Data represent means ± SD of 3 independent experiments. Different letters denote statistically significant differences between extracts (Tukey test, $p < 0.05$). n.d. non detected.

Table S2. Characteristics of standards analyzed by CZE at 280 nm.

Standars	Structure	Concentration (mM)	Migration time (min)	Absorption spectrum (280 nm)
Caffeine		5.15	3.100	
Melatonin		0.43	2.552	
Trigonellin hydrochloride		1.44	2.598	
Genistein		3.70	3.556	
Glycitein		0.88	4.158	

Catechin		0.86	4.318	
Cyanidin-3-O-glucoside chloride		2.06	5.500	
Procyanidin B2		0.43	4.672	
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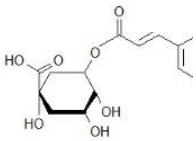
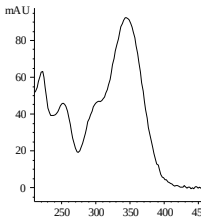
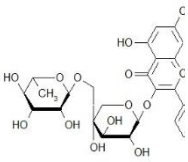
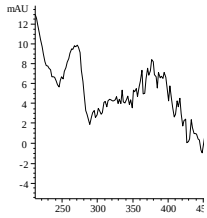
Chlorogenic acid		0.71	4.688	
Rutin hydrate		1.64	5.470	

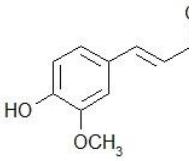
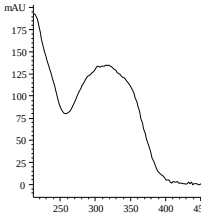
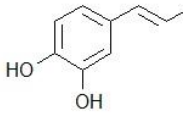
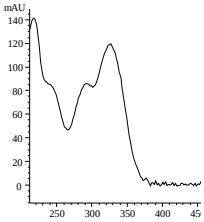
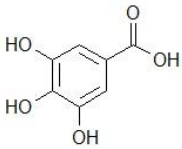
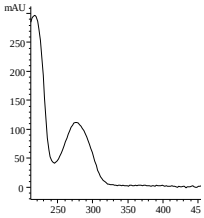
Ferulic acid		5.15	6.877	
Cafeic acid		5.55	9.940	
Galic acid		5.88	11.047	

Table S3. Effect of 24 h (for IEC-6 cells) and 48 h (RAW 264.7 cells) treatment with noted concentrations of extracts and yogurts determined by the MTT assay. Extract concentrations correspond to the amount of extract in each yogurt. Control, non-treated cells. DMSO (50%) was used as a death control.

Extracts	**Cell viability (%)**		**Yogurts**	**Cell viability (%)**	
(µg/ml)	**IEC-6**	**RAW 264.7**	**(mg/ml)**	**IEC-6**	**RAW 264.7**
Control	100.0 ± 0.0 ^{def}	100 ± 0.0 ^{cde}	Control	100.0 ± 0.0 ^{b}	100 ± 0.0 ^{f}
Death control	6.6 ± 1.8 ^{a}	7.1 ± 4.2 ^{a}	Death control	6.6 ± 1.8 ^{a}	6.5 ± 4.5 ^{a}
			Control		
			1	95.1 ± 4.6 ^{b}	94.5 ± 10.4 ^{f}
			10	95.7 ± 8.0 ^{b}	91.8 ± 12.1 ^{ef}
			100	85.9 ± 12.3 ^{b}	50.5 ± 2.6 ^{bc}
Coffee cascara			*Coffee cascara*		
10	95.2 ± 5.2 ^{de}	82.4 ± 10.6 ^{bcd}	1	98.2 ± 3.0 ^{b}	95.6 ± 13.3 ^{f}
100	90.4 ± 7.8 ^{cde}	78.5 ± 3.7 ^{bc}	10	94.1 ± 8.5 ^{b}	85.6 ± 21.7 ^{def}
1000	92.9 ± 7.7 ^{cde}	67.4 ± 9.8 ^{b}	100	83.0 ± 6.6 ^{b}	45.3 ± 2.0 ^{bc}
Coffee silverskin			*Coffee silverskin*		
4	109.1 ± 4.7 ^{ef}	91.5 ± 10.9 ^{bcd}	1	99.9 ± 5.7 ^{b}	92.4 ± 10.0 ^{f}
40	98.7 ± 4.8 ^{def}	80.6 ± 2.4 ^{bcd}	10	94.5 ± 10.2 ^{b}	75.5 ± 12.2 ^{cdef}
400	99.0 ± 2.8 ^{def}	69.8 ± 7.3 ^{b}	100	88.1 ± 9.3 ^{b}	41.5 ± 4.5 ^{b}
Grape pomace			*Grape pomace*		
5	91.7 ± 3.7 ^{cde}	105.0 ± 9.5 ^{cdef}	1	96.2 ± 6.8 ^{b}	95.1 ± 9.0 ^{f}
50	68.3 ± 16.1 ^{bcd}	87.6 ± 8.2 ^{bcd}	10	93.2 ± 5.5 ^{b}	92.5 ± 4.2 ^{f}
500	61.2 ± 14.4 ^{bc}	82.3 ± 17.1 ^{bcd}	100	85.4 ± 9.3 ^{b}	57.5 ± 12.5 ^{bcd}
Grape skin			*Grape skin*		
5	100.9 ± 10.6 ^{def}	106.8 ± 0.2 ^{cdef}	1	94.8 ± 10.9 ^{b}	95.7 ± 13.6 ^{f}
50	112.4 ± 11.0 ^{ef}	99.4 ± 6.0 ^{cde}	10	89.7 ± 13.0 ^{b}	92.6 ± 11.6 ^{f}
500	116.0 ± 39.3 ^{ef}	132.5 ± 15.3 ^{f}	100	79.4 ± 6.7 ^{b}	60.3 ± 13.6 ^{bcde}
Grape seed			*Grape seed*		
5	90.0 ± 6.8 ^{cde}	108.6 ± 11.6 ^{def}	1	86.3 ± 16.0 ^{b}	92.2 ± 7.9 ^{ef}
50	37.3 ± 10.9 ^{ab}	85.7 ± 1.7 ^{bcd}	10	90.9 ± 7.4 ^{b}	88.1 ± 10.4 ^{def}
500	131.0 ± 23.4 ^{f}	125.9 ± 19.1 ^{ef}	100	82.4 ± 3.9 ^{b}	50.5 ± 5.0 ^{bc}

Data represent means ± SD of 3 independent experiments. Different letters denote statistically significant differences between extracts or yogurts (Tuckey test, $p < 0.05$).

Table S4. Effect of 24 h (for IEC-6 cells) and 48 h (RAW 264.7 cells) treatment with noted concentrations of yogurt digests on cell viability determined by the MTT assay. Control, non-treated cells. DMSO (50%) was used as a death control.

Yogurt digests (mg/ml)	**Cell viability (%)**	
	IEC-6	**RAW 264.7**
Control	100.0 ± 0.0 [d]	100.0 ± 0.0 [b]
Death control	2.8 ± 0.2 [a]	6.2 ± 2.2 [a]
Control		
0.01	100.3 ± 2.0 [d]	107.0 ± 0.3 [b]
0.1	89.6 ± 10.5 [cd]	105.7 ± 9.2 [b]
1	64.9 ± 3.3 [b]	92.0 ± 13.6 [b]
Coffee cascara		
0.01	93.3 ± 4.9 [cd]	108.8 ± 7.9 [b]
0.1	81.7 ± 8.0 [c]	102.9 ± 17.2 [b]
1	57.7 ± 10.7 [b]	86.0 ± 14.6 [b]

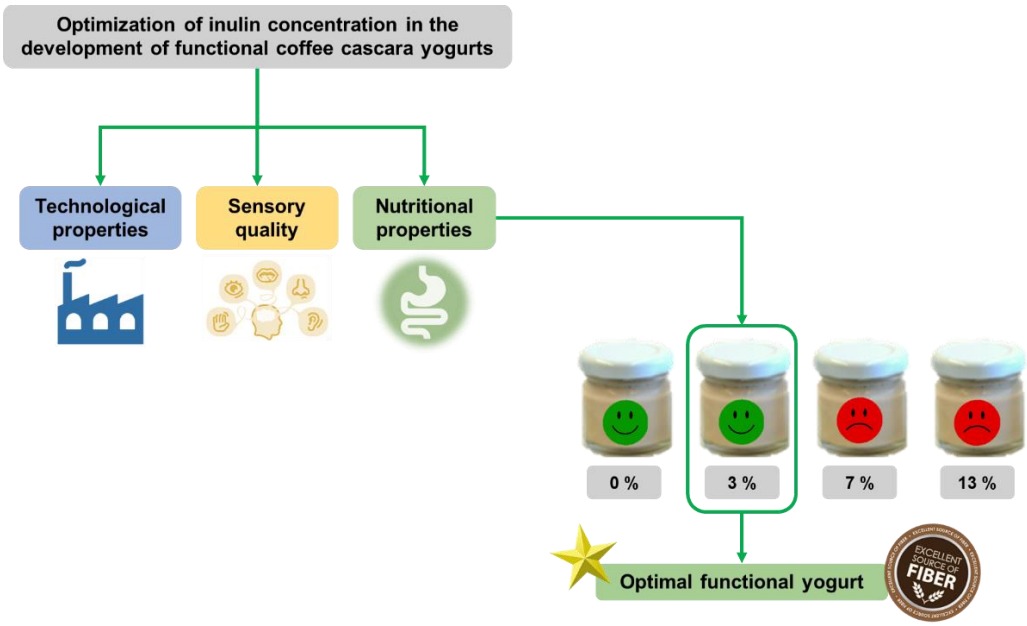
Data represent means ± SD of 3 independent experiments. Different letters denote statistically significant differences between extracts or yogurts (Tuckey test, $p < 0.05$).

CHAPTER 4

A multidisciplinary approach for the optimization of the quality and health benefits of yogurts containing coffee cascara and inulin

In this chapter the secondary gastrointestinal effects associated with dietary fiber consumption, as well as the satiety effect and sensory properties of coffee cascara yogurts were evaluated in a crossover nutritional trial in healthy adults.

Iriondo-DeHond, M., Iriondo-DeHond, A., Herrera, T., Fernández-Fernández, A.M., Sorzano, C.O., Miguel, E. & del Castillo, M.D., 2019. A multidisciplinary approach for the optimization of the quality and health benefits of yogurts containing coffee cascara and inulin.



A multidisciplinary approach for the optimization of the quality and health benefits of yogurts containing coffee cascara and inulin

Maite Iriondo-DeHond [12], Amaia Iriondo-DeHond [2], Teresa Herrera [2], Adriana Maite Fernández-Fernández [3], Carlos Oscar Sorzano [4], Eugenio Miguel [1], María Dolores del Castillo [2]*

1 Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Alcalá de Henares, Spain.

2 Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM), Madrid, Spain.

3 Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, Montevideo, Uruguay.

4 Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain.

Abstract

Purpose: To optimize the concentration of inulin (0, 3, 7 and 13%) in the development of functional coffee cascara yogurts attending to its effects on the technological, sensory and nutritional properties of yogurts.

Methods: Four test yogurts were produced: cascara yogurt without dietary fiber (Y0), and containing 3, 7 and 13% dietary fiber (Y3, Y7 and Y13; respectively). The technological properties of inulin as an ingredient in coffee cascara yogurts were evaluated in terms of syneresis and instrumental texture improvement. The sensory and nutritional effects of inulin were analyzed in a blind crossover design, where 45 healthy adults were allocated in balanced order to receive one of the yogurt treatments in acute conditions. Test days were separated with a minimum of 48 h. Ratings on sensory acceptance, satiety, gastrointestinal tolerance and stool frequency were measured. Surveys were carried out digitally in each participants cellphone.

Results: Cascara addition lowered the yogurt's firmness and consistency, which was recovered by inulin addition. Yogurt containing 13% of inulin achieved significantly higher overall acceptance, texture and taste scores than the control yogurt without inulin ($p < 0.001$). The yogurt containing 3% of inulin presented similar gastrointestinal

tolerance to the control. However, 7% and 13% of inulin produced significant ($p < 0.05$) bloating and flatulence compared to the control. Appetite ratings were not significantly affected by the acute intake of the different yogurts.

Conclusions: The cascara yogurt containing 3% dietary fiber, which could be labelled as "source of fiber", was identified as the optimal product from a technological and nutritional point of view.

Keywords: coffee byproduct; coffee cascara; gastrointestinal-tolerance; nutritional trial; satiety; organoleptic properties

1. Introduction

Suboptimal diets are one of the leading risk factors for the prevalence of noncommunicable diseases. Coronary heart disease, stroke, type 2 diabetes and obesity pose a substantial health and economic burden, causing 17 million deaths worldwide [1]. Because the epidemic of diet-related cardiometabolic diseases are forecast to increase in coming years, government measures are emerging for improving the nutritional quality of the food supply. These measures are directed at actors in the food supply chain (producers, processors and retailers) to reduce the levels of critical nutrients such as salt, sugar or fat, and promote functional and healthier foods [2, 3]. Dairy products are one of the food groups for which reformulation policies are prioritized due to the excessive amount of added sugars [2].

Functional foods are those that have a potentially positive effect on health beyond basic nutrition, promoting optimal health conditions and reducing the risk of non-communicable diseases [4]. Several authors have stated the need to develop functional foods with a multidisciplinary approach that combines principles from sensory science, nutrition physiology, ingredient technology and texture design [4, 5]. Following this multidisciplinary approach, we proposed the development of functional yogurts including ingredients (coffee cascara and inulin) involved in the metabolism of carbohydrates.

Coffee cascara is a food byproduct from the coffee industry, which has shown in vitro antioxidant, antidiabetic and anti-inflammatory properties [6]. Inulin is an indigestible carbohydrate that induces a lower blood glucose rise after its consumption compared to sugar-containing foods [7], and has been widely used in dairy products both as a texturizer and sweetener [8]. However, inulin consumption may have some dose-related undesirable effects due to their natural osmotic potential and/or excessive fermentation [9]. Therefore, inulin concentrations should be based on a compromise between their nutritional and technological properties (improving sweetness and mouthfeel) without producing secondary gastrointestinal effects. The aim of this study was to optimize the concentration of inulin attending to its effects on the technological, sensory and nutritional properties in yogurts containing coffee cascara and dietary fiber. For this purpose, a crossover nutritional trial was conducted to evaluate the gastrointestinal tolerance, satiety and sensory acceptance of yogurts containing coffee cascara extract and different doses of inulin.

2. Materials and Methods

2.1. Test foods

Test yogurts were made at the Institute of Food Research (CIAL-CSIC) using UHT whole cow milk (Unicla, Feiraco, Spain), inulin (Orafti®GR, Beneo, Belgium) and

coffee cascara from Arabica species and Colombian origin (Supracafe, Spain). Cascara extract was produced as described in the patent WO 2013/004873 [10].

Four test yogurts were produced: control cascara yogurt without dietary fiber (Y0), cascara yogurt with 3% dietary fiber (Y3), cascara yogurt with 7% dietary fiber (Y7) and cascara yogurt with 13% dietary fiber (Y13). Cascara extract was employed as an antidiabetic ingredient due to its inhibitory properties against the enzyme α-glucosidase (IC50 = 0.70 ± 0.10 mg/mL) (Fig.S1). The α-glucosidase inhibitory capacity of coffee cascara extract was analyzed following the methodology described by Berthelot & Delmotte [11] and Geddes & Taylor [12] with modifications [13].

For the test yogurt elaboration, milk (3.6% fat, 3.1% protein, 4.8% sugar) was put in a vat and heated up to 45ºC to inoculate the starter culture YO-MIX 300 (Danisco), containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. Inulin was added at 3 g/100 mL, 7 g/100 mL or 13 g/100 mL in the yogurts containing soluble dietary fiber. All yogurt types contained cascara extract at a final concentration of 10 mg/mL. Extract concentration was selected using a focus group of 10 volunteers who tested yogurts containing cascara extract within a range of 3 – 15 mg cascara/mL yogurt. After the addition of the ingredients, milk was stirred and separated into pots of 125 g. Individual pots were incubated at 45ºC during 5h until pH reached approximately 4.5. Samples were stored at 4ºC. Yogurt samples for texture and syneresis analysis were elaborated separately in three independent sets.

2.2. Technological characterization of test yogurts

2.2.1. Texture characterization of yogurts

The impact of the addition of coffee cascara extract and inulin in the yogurt's texture was analyzed using a TA.XTplus Texture Analyzer (Stable Micro Systems, UK). A back-extrusion test was carried out using a cylindrical stainless-steel probe (35 mm diameter). Yogurts for texture analysis were made directly into cylindrical containers (50 mm diameter and 50 mm high) so that their solid structure would be kept intact prior to the texture analysis. The probe penetrated the sample to a depth of 10 mm at 1 mm/s. Firmness (N) and consistency (Ns) were calculated from the deformation curves using the Exponent software (Stable Micro Systems, UK). A control yogurt without coffee cascara and inulin was included in this analysis. Measurements were performed in triplicate.

2.2.2. Syneresis

Yogurt syneresis was calculated by centrifugation [14]. Results were expressed in percentage according to the following equation:

$$\text{Syneresis (\%)} = [\text{expelled whey (g)} / \text{yogurt mass (g)}] \times 100$$

2.3. Nutritional study

2.3.1. Participants and ethical aspects

Forty-five healthy participants were recruited through advertising at the Autonomous University of Madrid and surrounding areas. Inclusion criteria included apparently healthy participants, male or female, age ≥ 18 and ≤ 60 years old. The exclusion criteria were: pregnancy or breastfeeding, having food allergies, lactose intolerance, milk protein allergy, and diagnosed gastrointestinal disorders/diseases.

This study was granted a favorable ethical opinion from the Spanish National Council Ethics Committee (reference: 034/2017) and was registered under http://clinicaltrials.gov as NCT03539146. The study was conducted in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki and in accordance with Good Clinical Practice guidelines. All participants gave written informed consent to participate in the study and they were aware of the possibility of withdrawing from the study at any time they desired.

2.3.2. Study design and protocol

This study used a blind, crossover design including four treatments of yogurts containing coffee cascara extract and different doses of inulin (0, 3, 7 and 13%). Four sequences of intervention groups were used following a completely balanced Latin square design to avoid first-order carryover effects.

Over a period of 2 weeks, each volunteer visited the test facility on four occasions, with a minimum of 48-hour washout period between visits. Participants were asked not to change their dietary patterns or physical activity routine for the duration of the trial. On each test day, participants were asked to arrive at the Center of Molecular Biology cafeteria at 08.45 in fasting conditions to have a standardized breakfast. The standardized breakfast (300 Kcal) consisted on a 40 g toasted white bread baguette with 8 mL of olive oil and salt, 150 mL fresh orange juice and 200mL of tea or coffee. At 11.15 a test yogurt was given to each participant at the Institute of Food Research (CIAL-CSIC) together with a glass of water (150 mL). Participants were given the choice to work on a laptop at the Institute's facilities or at their own facility, as all volunteers worked on campus. However, they were instructed not to eat or drink between meals, with the exemption of small amounts of water.

Participants completed a baseline appetite rating at 09.00 prior to consumption of breakfast and were given 15 minutes to eat it. Questions on satiety were then asked every 30 minutes until the consumption of the yogurt at 11.15. Participants had 15 minutes to eat the test yogurt (125 g) and respond to a sensory acceptance questionnaire of the product. After consumption of the test yogurt participants continued to answer questions on satiety every 30 minutes until lunch time (13.30). At that time, participants were also

asked to answer a gastrointestinal symptom questionnaire (2-hours after test yogurt ingestion). Gastrointestinal symptoms were also evaluated with an online survey 24h after yogurt ingestion.

2.3.3. Sensory analysis

Participant's acceptance of test yogurts was assessed using a 9-point hedonic scale ranging from "1-dislike extremely" to "9-like extremely". The attributes evaluated included "overall liking", "visual appearance", "smell", "texture" and "taste". Data were collected on a 5-point just-about-right (JAR) scale to measure the appropriateness of the level of the following attributes: "creaminess", "sweetness", "vegetal or fruity flavor" and "milky flavor". The JAR scale ranged from 1 ("not enough at all") to 5 ("Far too much"). Liking and JAR data were used for a penalty analysis to study the relation between the rankings on the JAR scale and the results in the liking scores for the different attributes.

2.3.4. Appetite ratings

Subjective appetite ratings were measured electronically on each of the participants cell phone with the use of 100 mm VAS (RedJade Sensory Software). Participants were asked to set alarms on their cell phones at the indicated times and answers were checked online at real time. The appetite rating profile included measures of hunger, satiety, fullness, thirst and desire to eat something fatty, salty, sweet or savory. The scale was anchored at 0 mm ("nothing at all") and at 100 mm ("a large amount").

2.3.5. Gastrointestinal wellbeing measurements

Gastrointestinal symptoms were assessed 2 and 24-hours after test yogurt intake. Participants were asked to compare their symptoms to how they normally felt in a scale from 0 to 3 [15]: "0-no or habitual occurrence of symptom", "1-slightly more than usual", "2-much more than usual" and "3-exceptionally more than usual". The gastrointestinal profile included ratings on abdominal bloating, nausea, abdominal pain, flatulence, diarrhea, constipation and stomach rumbling. Individual gastrointestinal scores for each symptom were collected and the total score of gastrointestinal symptoms was calculated as the sum of the individual scores for each treatment. Participants were also asked to record the stool frequency and consistency in accordance with the Rome III clinical designation (constipation, normal, or diarrhea) after 24 hours.

2.4. Statistical analysis

Minimum sample size (n = 34) was estimated through a power analysis for the detection of 0.5 variation in gastrointestinal symptoms with a power of 80% and alpha = 0.05. A one-factor ANOVA with Tukey's test was used to analyze textural differences among yogurt samples. Randomization sequences were analyzed using one-factor analysis of

variance to examine whether there was evidence of carry-over effects among treatments. A Chi-square test was used to compare the frequency of gastrointestinal symptoms, stool frequency and stool consistency types associated to the ingestion of the different yogurt treatments to those of the control. These analyses were performed using R software version X. A two-way repeated measures ANOVA with Tukey's test was used to analyze satiety scores, with time as the within-subject factor. Satiety statistical analyses were performed using IBM SPSS Statistics version 24. Hedonic data was analyzed with a non-parametric test as data were not normally distributed. A Friedman test followed by multiple pairwise comparisons using Nemenyi's procedure was used for determining differences between treatments. Results from the JAR and liking surveys were used to determine the drop in overall liking associated with a deviation from the JAR for each attribute. Sensory analyses were conducted in XLStat-Sensory version 2018.6.

3. Results

3.1. Technological characterization of yogurts

3.1.1. Texture profile of yogurts containing cascara extract and dietary fiber

The use of cascara extract in yogurt produced a significant decrease ($p < 0.05$) in the yogurt's firmness and consistency (1.11 ± 0.16 N and 9.41 ± 1.26 Ns, respectively) compared to the control (1.71 ± 0.21 N and 14.11 ± 1.36 Ns, respectively) (Fig. 1a). The addition of inulin in yogurts containing cascara extract helped recover the yogurt's original texture. No differences were observed in firmness and consistency between the control yogurt without cascara extract and yogurts containing cascara extract and inulin at concentrations of 3, 7, 13 and 17%.

3.1.2. Yogurt syneresis

The addition of coffee cascara extract did not result in an increase of yogurt syneresis (Fig. 1b). Increasing doses of inulin decreased yogurt syneresis, which was significantly lower ($p < 0.001$) in yogurts containing 13% and 17% of inulin compared to the rest of the yogurt formulations.

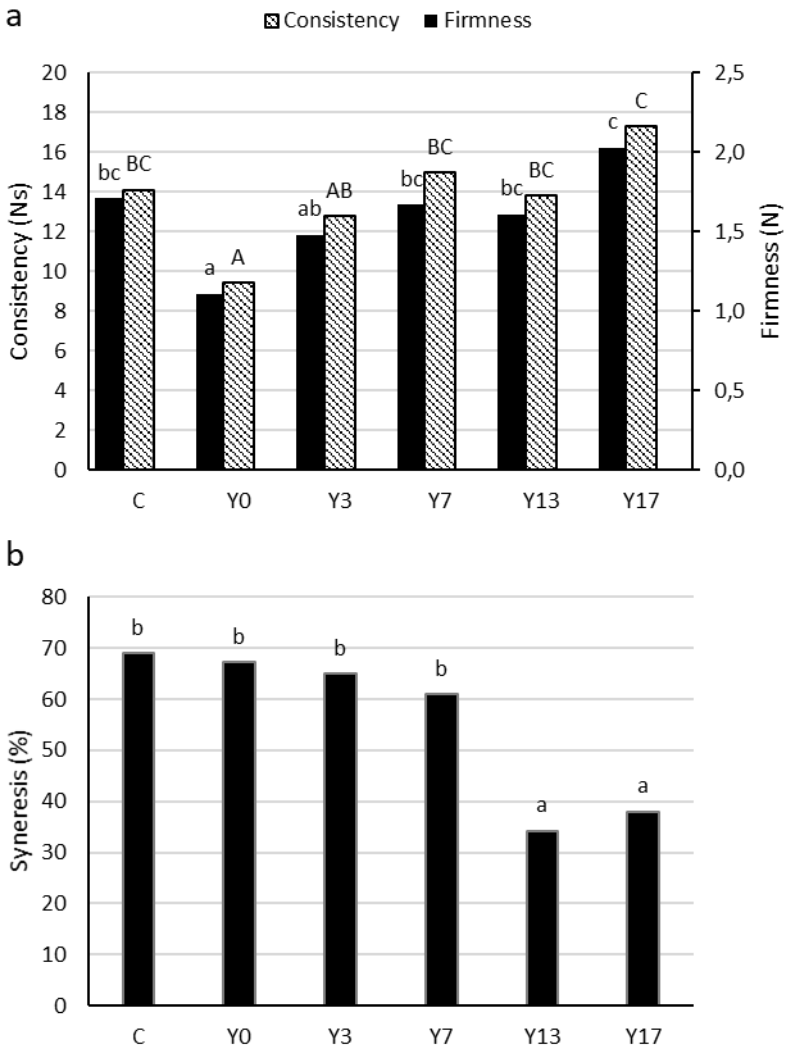


Fig. 1 Instrumental firmness (N) and consistency (Ns) of a control yogurt (C), yogurt with cascara extract (Y0), yogurt with cascara extract and 3% inulin (Y3), yogurt with cascara extract and 7% inulin (Y7), yogurt with cascara extract and 13% inulin (Y13), yogurt with cascara extract and 17% inulin (17).

3.2. Sensory quality and gastrointestinal effects of yogurt intake

3.2.1. Participants

Forty-six participants were included in the study, 26 females and 20 males. One participant withdrew from the study due to severe gastrointestinal symptoms. The participants ranged in age from 20 to 57 years with a median of 28.5 years. The participants had a median BMI of 22.8, with 4 (9%) underweight (BMI < 18.5), 28 (62%) normal weight (BMI ≥ 18.5 and < 25), 11 (24%) over- weight (BMI ≥ 25 and < 30), and 2 (4%) obese (BMI ≥ 30).

3.2.2. Sensory quality

Results from the hedonic test are show in Table 1. Overall liking was significantly higher ($p < 0.001$) in Y13 than in Y0. Other parameters influenced by the addition of inulin were texture and taste. Texture was significantly better accepted in Y13 ($p < 0.001$) compared to the rest of the yogurt formulations. Y13 also achieved significantly higher scores in taste ($p < 0.05$) than Y0 and Y3. JAR scale results showed that less than 65% of the consumers stated that "creaminess", "sweetness" and "fruity/vegetable flavor" were in the ideal-JAR point for all yogurt treatments (Fig. 2). However, increasing doses of inulin raised the number of respondents in the JAR point for "creaminess" and "sweetness". Over 65% of the participants rated "lactic flavor" of Y3, Y7 and Y13 as JAR. The penalty analysis showed the mean drop in liking scores for the attributes that had a significant negative effect ($p < 0.05$) and an occurrence higher than 20% of cases. These parameters identified that too little "lactic flavor" produced a significant ($p < 0.01$) mean drop in overall liking of 1.05 in Y0 and of 1.13 in Y13.

Table 1 Overall liking and acceptability of individual attributes evaluated by the participants (n = 45) for each yogurt treatment.

	Y0	Y3	Y7	Y13
Overall liking	5.47 ± 1.25^{a}	5.82 ± 1.23^{ab}	5.78 ± 1.39^{ab}	6.44 ± 1.23^{b}
Smell	6.42 ± 1.05^{a}	6.4 ± 1.03^{a}	6.6 ± 1.45^{a}	6.467 ± 1.11^{a}
Appearance	6.07 ± 1.21^{a}	5.91 ± 1.35^{a}	5.89 ± 1.51^{a}	6.47 ± 1.39^{a}
Texture	4.89 ± 1.60^{a}	5.31 ± 1.44^{a}	5.69 ± 1.65^{a}	6.76 ± 1.41^{b}
Taste	5.2 ± 1.47^{a}	5.38 ± 1.55^{a}	5.71 ± 1.61^{ab}	6.04 ± 1.69^{b}

Values are represented as mean ± SD.

Values with different letters are significantly different *($p < 0.05$).*

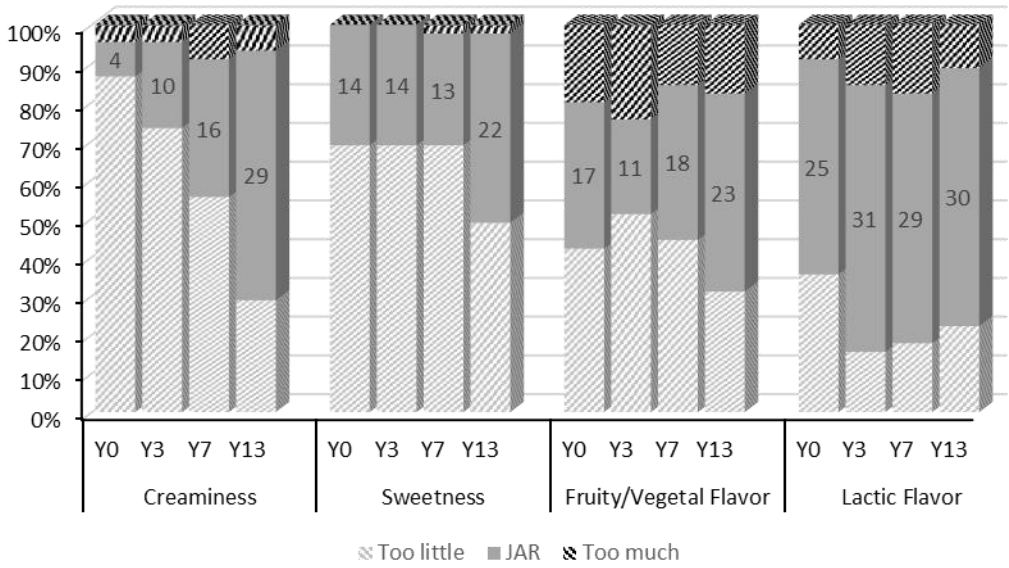


Fig. 2 Frequency distribution in percentage of consumer responses for Just-About-Right scores for each dimension (Too little, JAR, Too much) and each attribute. The number of consumers out of the total (n = 45) that rated an attribute as "Just About Right" is indicated in each column.

3.2.3. Appetite ratings

VAS ratings for hunger and fullness are shown in Fig. 3. No significant differences were found among the cascara yogurt treatments with increasing doses of inulin concerning ratings of hunger, satiety, fullness, thirst and desire to eat something fatty, salty, sweet or savory at the different time points.

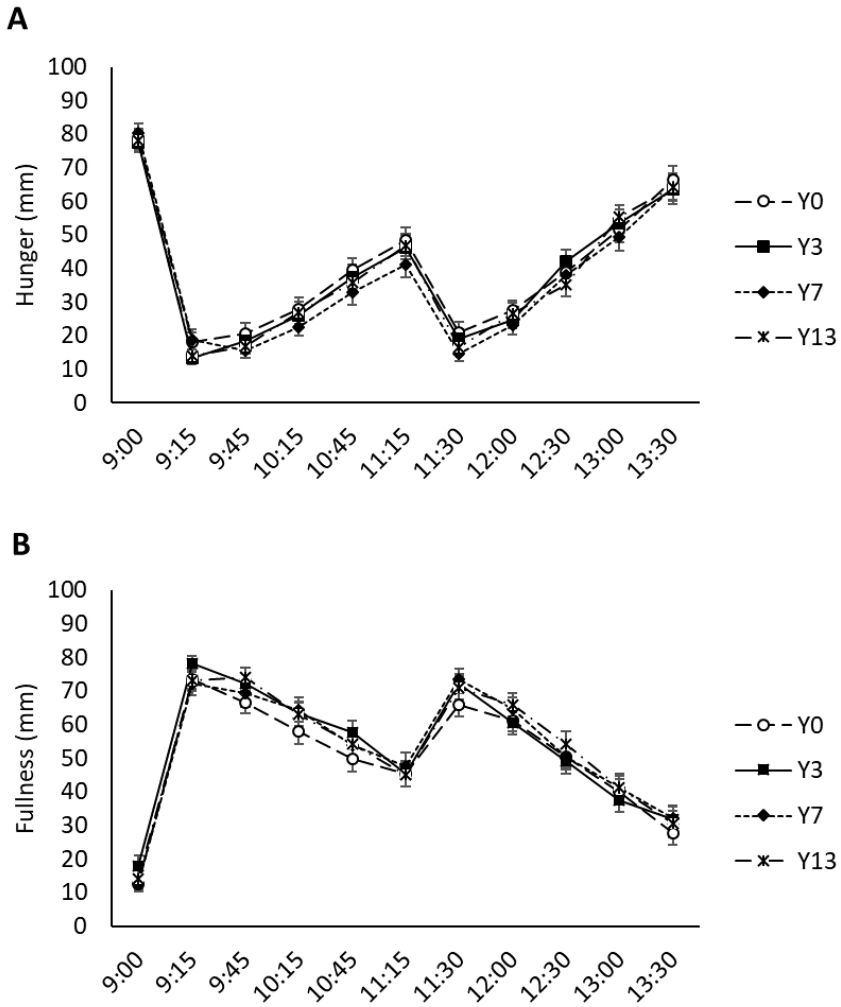


Fig. 3 VAS scores of hunger (a) and fullness (b) rated from the morning meeting in fasting conditions before the standard breakfast (9:00 h) to prior to lunch time (13:30 h).

3.2.4. Gastrointestinal Tolerance

Randomization sequences were not significantly different from one another *($p > 0.05$)* using one-factor analysis of variance indicating there was no evidence of carry-over effects among treatments. Occurrence of gastrointestinal symptoms was only reported between 2h and 24h after treatment intake (Table 2). Bloating was the most experienced symptom, with 49% and 58% of participants reporting occurrence in Y7 and Y13. Flatulence was the second most experienced symptom. Most of the participants who experienced these reported only mild occurrences, as the individual symptom scores remained in a low range (0.58 – 0.82 out of 3). Consumption of Y7 significantly

increased the occurrence of bloating ($p < 0.05$) and flatulence ($p < 0.01$) compared to Y0. In relation to Y13, a significant increase in the occurrence of bloating ($p < 0.01$), flatulence ($p < 0.01$), abdominal pain ($p < 0.05$) and gastrointestinal rumbling ($p < 0.05$) was observed compared to Y0. The consumption of Y3 did not significantly increase ($p > 0.05$) the occurrence of individual gastrointestinal symptoms compared to Y0. The total gastrointestinal symptom scores of yogurt treatments increased 0.18, 1.07 and 1.62 in Y3, Y7 and Y13, compared to the basal Y0 total score.

Table 2 Frequency of occurrence and total and individual scores of gastrointestinal symptoms in the 2 and 24 h following consumption of the yogurt treatments in healthy adults (n = 45).

	Yogurt formulation			
	Y0	Y3	Y7	Y13
Bloating				
No symptoms	31	32	23	19
More than usual	13	11	13	16
Much more than usual	1	2	9	9
Extremely more than usual	0	0	0	1
Significance	-	*ns*	*	**
Individual score	0.33 ± 0.52	0.33 ± 0.56	0.69 ± 0.79	0.82 ± 0.83
Nausea				
No symptoms	42	43	42	45
More than usual	2	2	3	0
Much more than usual	1	0	0	0
Extremely more than usual	0	0	0	0
Significance	-	*ns*	*ns*	*ns*
Individual score	0.09 ± 0.36	0.04 ± 0.21	0.07 ± 0.25	0.00 ± 0.00
Flatulence				
No symptoms	38	34	25	24
More than usual	7	9	14	14
Much more than usual	0	2	6	5
Extremely more than usual	0	0	0	2

Significance	-	*ns*	**	**
Individual score	0.16 ± 0.37	0.29 ± 0.55	0.58 ± 0.72	0.67 ± 0.85
Abdominal pain				
No symptoms	41	38	38	32
More than usual	4	6	6	11
Much more than usual	0	1	1	2
Extremely more than usual	0	0	0	0
Significance	-	*ns*	*ns*	*
Individual score	0.09 ± 0.29	0.18 ± 0.44	0.18 ± 0.44	0.33 ± 0.56
Diarrhea				
No symptoms	41	42	39	38
More than usual	4	3	4	6
Much more than usual	0	0	2	1
Extremely more than usual	0	0	0	0
Significance	-	*ns*	*ns*	*ns*
Individual score	0.09 ± 0.37	0.07 ± 0.25	0.18 ± 0.49	0.18 ± 0.44
Constipation				
No symptoms	40	42	42	41
More than usual	5	2	2	3
Much more than usual	0	1	1	1
Extremely more than usual	0	0	0	0
Significance	-	*ns*	*ns*	*ns*
Individual score	0.11 ± 0.32	0.09 ± 0.36	0.09 ± 0.36	0.11 ± 0.38
GI rumbling				
No symptoms	39	37	34	28
More than usual	6	8	9	11
Much more than usual	0	0	2	6
Extremely more than usual	0	0	0	0

Significance	-	*ns*	*ns*	*
Individual score	0.13 ± 0.34	0.18 ± 0.39	0.29 ± 0.55	0.51 ± 0.73
Total score	1.00	1.18	2.07	2.62

Results are expressed as frequency of occurrence of individual gastrointestinal symptoms. Differences between the occurrence of individual gastrointestinal symptoms for the different yogurt samples were analyzed using a Chi-square test comparing the frequencies reported for Y3, Y7 and Y13 to those reported for the control Y0.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant

Stool frequency did not significantly increase ($p > 0.05$) within the 24 h following the intake of yogurts containing cascara extract and inulin (Table 3). The frequency of stools with a consistency similar to diarrhea slightly increased after consumption of yogurts containing higher concentrations of inulin (Y7 and Y13), although the difference was not significant compared to the frequency of diarrheic stools reported in the control yogurt.

Table 3 Stool frequency and consistency within 2 to 24 h following the consumption of the yogurt treatments in healthy adults (n = 45).

	Yogurt formulation			
	Y0	Y3	Y7	Y13
Stool frequency [1]	1.47 ± 0.79	1.36 ± 0.68	1.76 ± 1.07	1.51 ± 0.79
Significance	-	ns	ns	ns
Stool consistency [2]				
Constipation	3	2	3	2
Normal	32	35	30	28
Diarrhea	7	4	10	14
Significance	-	ns	ns	ns

[1] Stool frequency per participant is expressed as mean ± sd. Chi-square comparisons were made between relative frequencies of Y0 and the rest of the treatments (Y3, Y7, Y13).

[2] Stool consistency is expressed as the frequency reported for each stool consistency type for the different yogurt treatments. Differences between the occurrence of stool consistency types for the different yogurt samples were analyzed using a Chi-square test comparing the frequencies reported for Y3, Y7 and Y13 to those reported for the control Y0.

4. Discussion

The development of a coffee cascara functional yogurt was approached by a multidisciplinary perspective considering the technological properties of the food product, its nutritional composition and hedonic component. The selection of coffee cascara as the source of bioactive compounds was due to previous results from our research group in which coffee cascara showed in vitro antioxidant, antidiabetic and anti-inflammatory properties [6]. These properties were maintained once incorporated into the yogurt matrix and bioaccessible after *in vitro* digestion. Coffee cascara has been used as an ingredient in the American beverage industry. In Europe, coffee cascara is considered a novel food and must comply with Regulation (EU) 2015/2283 to achieve authorization by the European Food Safety Authority (EFSA) before it can be used in foods in the European market. Evidence on the functional potential (antioxidant and antidiabetic properties) and safety (pesticide, mycotoxin, acrylamide and acute toxicity experiments) of coffee cascara as a sustainable food ingredient is already being gathered [6, 16].

The addition of coffee cascara extract provided a unique flavor and significantly decreased ($p < 0.05$) the yogurt's firmness and consistency, which justified the technological use of inulin due to its texture modifying properties. Increasing concentrations of inulin significantly improved the yogurt's firmness and consistency. Addition of inulin also improved the yogurts syneresis, which refers to the serum release from the gel matrix and is regarded as a technological defect in set yogurts. Similar results on syneresis reduction and increased firmness were observed in set yogurts with increasing doses of inulin (0 to 4%) [17]. The physicochemical properties of inulin are linked to their degree of polymerization (DP) (average DP in this study ≥ 10), which affects its solubility, viscosity, capability of gel formation, gel strength and interaction with other food components [18, 19].

The sensory analysis helped to evaluate the technological properties of inulin as a texture and flavor enhancer. The overall liking of the formulated yogurts showed scores between 5.47 to 6.44. Y13 was the only yogurt formulation that showed significantly higher overall liking, texture and taste scores compared to the control. A product can be considered as potentially successful from a sensory standpoint when it receives a satisfactory evaluation for its attributes (scores ≥ 6 in a 9-point hedonic scale) [4]. In our study, only Y13 achieved scores higher than 6 for all its attributes. Increasing levels of inulin in the yogurts showed a positive tendency improving the acceptance scores for all attributes. JAR scales measured the appropriateness of the level of specific attributes and were used as guidance for product reformulation. The number of participants which rated "creaminess" as JAR increased with increasing doses of inulin, which confirmed the capacity of inulin for improving texture and mouthfeel perception. Only yogurts with 13% of inulin increased the number of participants rating an optimal sweetening

perception, although in general terms all yogurt formulations were considered "not sweet enough". The addition of 6% inulin to sheep yogurt produced similar results when evaluating the consistency and sweetness by JAR scales, showing an improvement in the product's consistency but not in the sweet flavor [20]. The hedonic scores for "taste" and JAR data on "fruity of vegetable flavor" in yogurts suggest that the unique flavor of coffee cascara, although unfamiliar, was well accepted. Results from the hedonic and JAR tests showed that Y13 was the best formulation from a sensory point of view, suggesting that texture improvement and sweet flavor from inulin promote acceptability of the coffee cascara.

From a nutritional point of view, inulin concentrations of 3% or 6% are necessary to achieve a "source of fiber" or "high in fiber" nutritional claim [21]. Also, concentrations between 3 to 8 g of inulin per portion are necessary to assure a bifidogenic shift in the composition of the colonic microbiota that could have beneficial effects for the human host [22]. General recommendations on daily consumption of inulin suggest that intakes below 10 g may cause mild gastrointestinal symptoms, and that intakes between 10 – 15 g of inulin per day would be tolerated by most individuals. Intakes of more than 20 g per day may increase the occurrence and severity of the symptoms [23]. However, when developing a novel functional food, it is necessary to evaluate its gastrointestinal acceptance as it is the limiting factor in the consumption of these compounds by consumers.

To our knowledge, this is the first time that the gastrointestinal tolerance of yogurt containing coffee cascara and different doses of inulin has been evaluated alone in acute conditions to simulate the action of eating a yogurt as a mid-day snack. The selection of the optimal inulin concentration for the development of functional cascara yogurts was based on the condition that no significant differences should be observed on the gastrointestinal tolerance between the control yogurt without inulin and the yogurts with inulin. Our results show that this threshold was only true for the yogurt containing 3% of inulin (Y3), which corresponded to 3.75 g of inulin per portion (125 g of yogurt) that would allow a "source of fiber" nutritional claim. Although this approach may appear conservative, it is better to identify the upper intake levels to minimize the risk of undesirable gastrointestinal effects while maximizing the potential health benefits of inulin consumption. This is because gastrointestinal symptoms affect the perception of the well-being of consumers and their acceptance of food products containing indigestible carbohydrates, which could put at risk their marketability. The addition of 7% inulin resulted in the significant occurrence of bloating and flatulence compared to the control. At higher doses (13%) more painful symptoms occurred such as abdominal cramps, also accompanied by bloating, flatulence and gastrointestinal rumbling. These results are in accordance with previous reports on the order of occurrence of individual gastrointestinal symptoms associated with increasing intake of indigestible carbohydrates [9]. Diarrhea is always the last intolerance symptom occurring with high

doses of indigestible carbohydrates. In our study, none of the yogurts containing inulin produced significant differences in stool frequency or in the occurrence of different stool consistencies compared to the control.

The occurrence of gastrointestinal symptoms may have been generally intensified in our study by the fact that inulin was added in a semisolid food matrix (yogurt) without the simultaneous intake of other foods which can help increase tolerance [24]. Yogurt may travel through the gastrointestinal tract and be absorbed relatively more quickly than more solid foods, which are generally better tolerated than liquid structures [25, 26]. Previous studies have reported that inulin intake in acute conditions was well tolerated at 5, 9 and 10 g when administered together with breakfast [27–29]. Also, studies which spread out the dose of fiber throughout the day also observed improved inulin tolerance [30]. Therefore, it is possible that cascara yogurts containing amounts of inulin above 3% could be better tolerated if the yogurt is incorporated as part of a meal.

Results on the appetite measures after consumption of the yogurt treatments showed that increasing concentrations of inulin in yogurt did not modify ratings on hunger, fullness, satiety, thirst and desire to eat something fatty, salty, sweet or savory. Similarly, previous studies showed no differences in hunger ratings of healthy young adults between yogurts with or without 6 g of inulin in a one-time consumption basis [32]. The effects of cascara yogurts with inulin on appetite ratings may be obtained in a long-term treatment. Two-weeks of repeated consumption of symbiotic yogurts containing 4g of inulin has shown to reduce reported energy intakes [33]. Also, eight-day consumption of yogurts containing 6 g of inulin significantly lowered “desire to eat” and “prospective food consumption” ratings [31].

5. Conclusion

This preliminary study manifests the need to work with a multidisciplinary approach in the development of functional foods. Overall, results suggest that consumers seek a creamy texture and sweet flavor in yogurts, which was best achieved in the cascara yogurt with 13% of inulin. As this formulation produced negative gastrointestinal symptoms, we considered that from a nutritional point of view the best dose of inulin was 3%, which may be combined with a low caloric sweetening, agent such as sugar alcohols, to improve its sensory acceptance.

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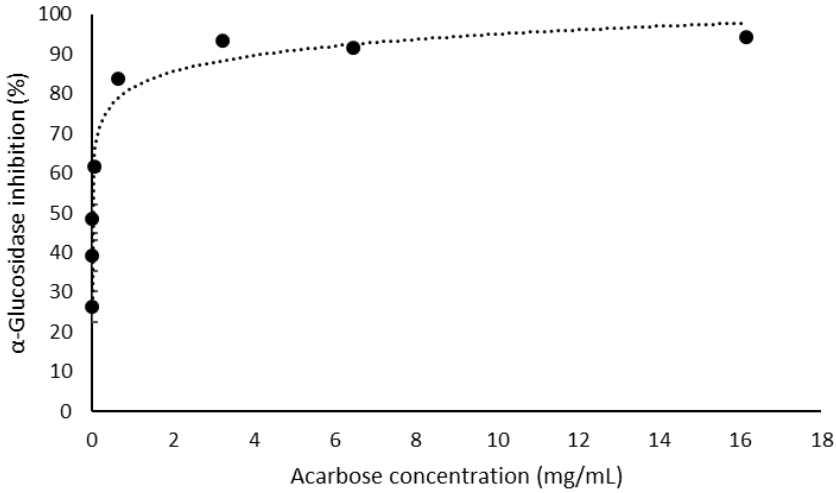
(a)



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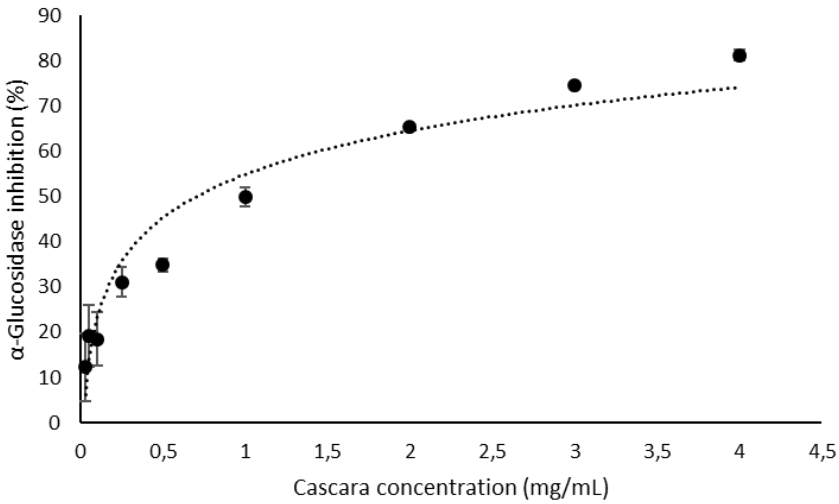


Fig. S1 Effect on α-glucosidase activity is represented by dose-response curves of (a) acarbose (0.06 µg/mL – 16 mg/mL); (b) Coffee cascara byproduct (0.01 – 4 mg/mL). Values represent mean ± standard deviation. This includes a duplicate of sample preparation and a triplicate of analysis.

GENERAL DISCUSSION

“ *If I had a world of my own, everything would be nonsense. Nothing would be what it is, because everything would be what it isn't. And contrary wise, what is, it wouldn't be. And what it wouldn't be, it would. You see?* ”

Lewis Carroll, Alice in Wonderland

1. New product development strategy, selection of byproducts as sources of novel ingredients and dairy matrix for their application

The present thesis followed the process described in Figure 1 to bring functional foods with health-promoting properties to the market. We focused on a novel dairy formulation without added sugars, which combined dietary fiber and glucoregulatory bioactive compounds for the prevention of diet-related chronic diseases. This formulation was motivated by the predominance of dairy products with excessive amounts of added sugars in the marketplace, which contribute to the development of metabolic disorders.

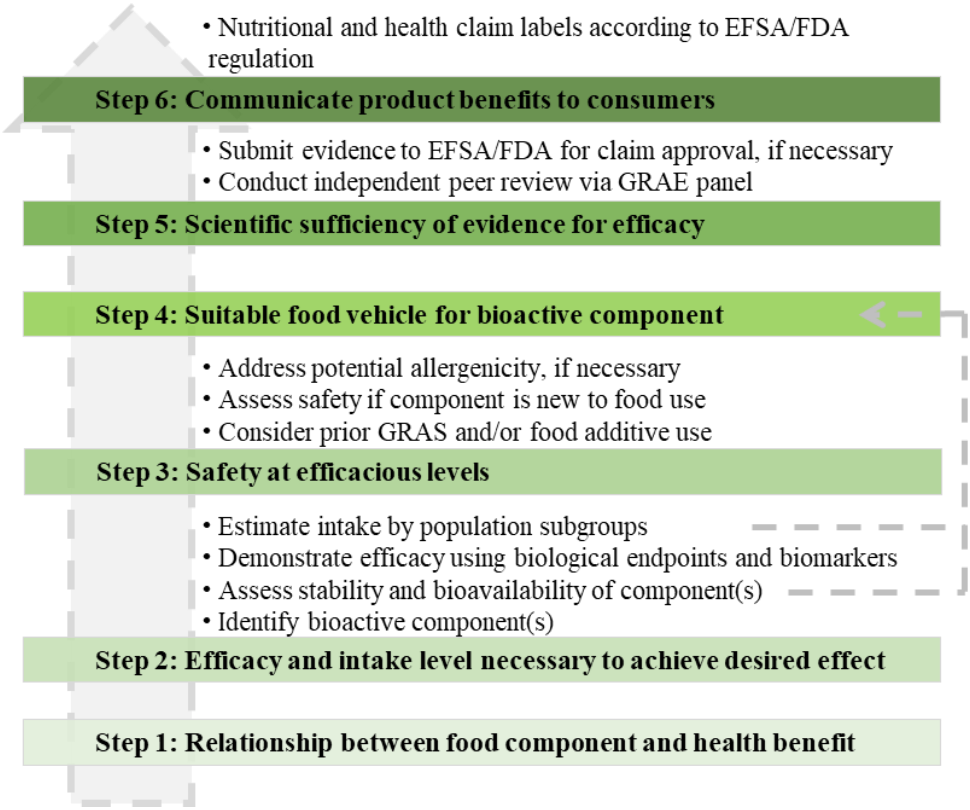


Figure 1. Steps for developing functional foods proposed by the Institute of Food Technologists (IFT), 2005. Figure adapted from the IFT Expert Report Panel on Functional Foods [1].

First, byproducts from the coffee and wine industry were selected as novel ingredients. This selection was based on three pre-established criteria: (I) existence of scientific evidence on target biological properties of the byproduct extracts, (II) familiarity of the byproducts to potentially facilitate their acceptance as ingredients, and (III) global abundance and environmental impact of byproduct generation.

Regarding the relationship between the food components and their potential health benefits, we examined the antioxidant, antidiabetic and anti-inflammatory properties of the byproduct extracts as a combination strategy for preventing diabetic complications (Figure 1, Step 1 and 2). These *in vitro* determinations were conducted as a preliminary assessment of their potential functionality as novel food ingredients. The biological properties were selected due to the relation between chronic hyperglycemia, oxidative stress and inflammation in diabetes pathobiology [2]. Results from the present thesis of the *in vitro* biological analyses of grape pomace, seed and skin extracts (*Chapter 3, Table S1*) confirmed their known antioxidant [3,4], antidiabetic [5,6] and anti-inflammatory [7,8] properties. These properties were attributed to the presence of cyanidin-3-O-glucoside chloride and catechin, which were detected by CZE in the present investigation (*Chapter 3, Figure 1*).

On the other hand, coffee cascara and silverskin extracts also presented the target biological properties. As coffee byproduct extracts have received less attention, the present thesis contributed to adding new information on their biological properties. To our knowledge, this study is the first to describe the *in vitro* antidiabetic properties of coffee cascara extract as an inhibitor of the α-glucosidase activity (*Chapter 3, Table S1*), the anti-inflammatory properties of coffee silverskin extract in cellular models (*Chapter 3, Figure 2*) and the antioxidant properties of coffee cascara and silverskin extracts in intestinal cells (*Chapter 3, Table 2*). In this case, the biological properties were attributed to the presence of chlorogenic acid, ferulic acid and caffeine (*Chapter 3, Figure 1*). This is in agreement with previous findings on the antidiabetic properties of pure caffeine and chlorogenic acid [9,10], and the anti-inflammatory properties of pure chlorogenic and ferulic acids [11,12]. As all coffee and wine-making byproduct extracts presented the target biological properties, they were all considered as suitable candidates for the development of functional foods for the prevention of metabolic disorders.

As the use of food byproducts as novel ingredients in other foods is reasonably new, there is a knowledge gap on their perception by consumers. Due to the lack of information of their potential acceptance, we based our selection of coffee and wine byproducts on the premises that consumers are familiar with the beverages and raw

materials, as coffee and wine are two of the most consumed beverages worldwide, and that consumers' have shown a positive perception between health and coffee [13] and grape derivatives [14], which is resembled by the increasing amounts of commercially available food supplements containing grape products or green coffee bean extracts. In addition, coffee and wine consumption has suffered a transformation from pure commodities to specialty products, which has been triggered by the connection between three food drivers that currently characterize consumers from specialty foods: pleasure, health and sustainability [15]. Therefore, these associations could potentially increase their acceptance compared to byproducts generated from other less common foods.

Finally, byproducts generated during coffee and wine production represent a global environmental issue. This may have motivated the use of wine-making byproducts as novel ingredients in dairy foods (*Chapter 1; Table 1*), which represented 19% of the total studies reviewed in the present thesis (Figure 2).

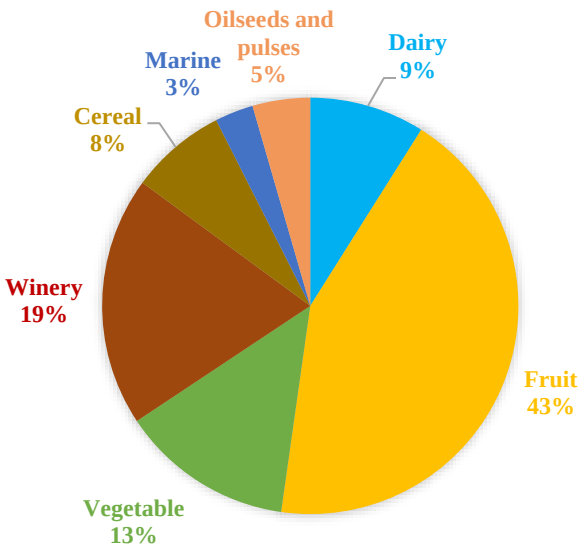


Figure 2. Percentage of published studies from 2000 to 2017 that have applied food byproducts from the different commodity groups in the development of novel dairy products.

Several food applications of coffee byproducts have also been developed to valorize these fractions and palliate their environmental impact. Examples include applications of coffee silverskin in novel beverages [16] or coffee silverskin and spent coffee grounds in bakery foods [17–19]. However, no applications were found of coffee byproducts in

dairy product development, which highlighted the opportunity for using these ingredients in dairy innovations. The use of coffee and wine-making byproducts not only contributes to sustainability from an environmental point of view, but it could potentially create future social and economic opportunities (Figure 3) [20].

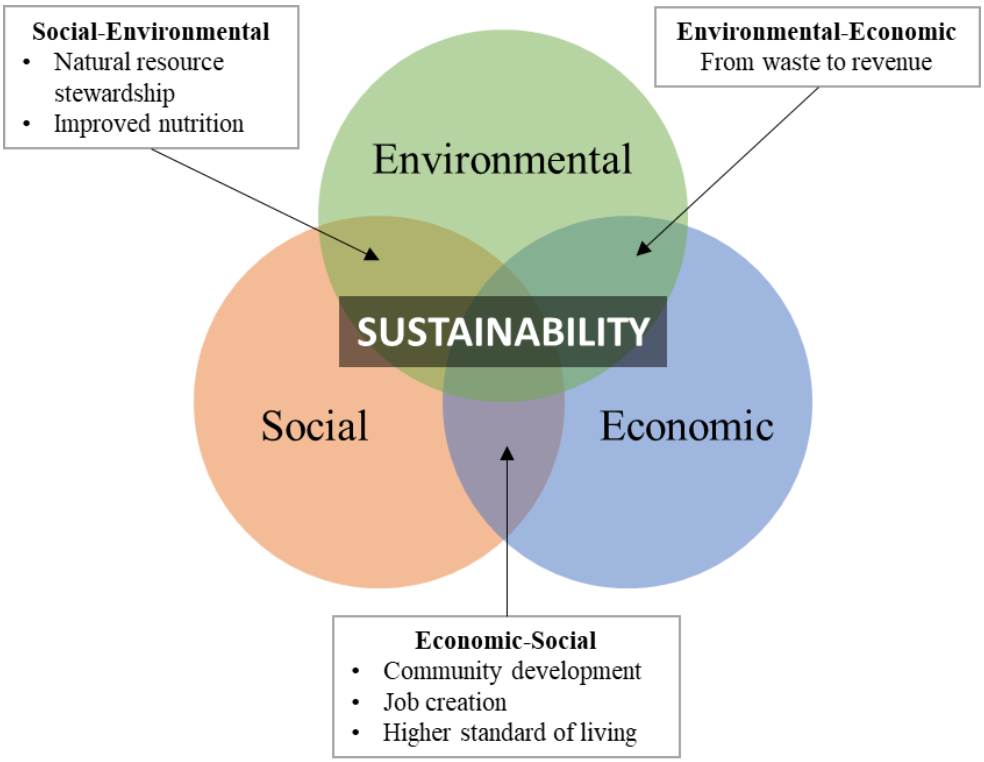


Figure 3. Sustainability representation by its environmental, social and economic components [20].

Food byproducts have also been used as sources of dietary fiber (*Chapter 1*, *Table 1*). However, we selected commercial soluble fibers derived from chicory root (inulin and FOS) due to their technical suitability in dairy product development [21], to their prebiotic effect [22], and to their role in the prevention of the target diseases of the thesis in relation to SCFA production and appetite control [23]. Moreover, the development of dairy foods containing inulin could lead to the health claim "maintenance of normal defecation by increasing stool frequency" when a minimum dose of 12 g of inulin is consumed per day. Other general health claims that could be applied according to

Regulation (EU) 1924/2006 include promotion of digestive health and support of a healthy and balanced digestive system [24].

In relation to the safety of the byproduct extracts (Figure 1, Step 3), previous research from our research group conducted the analyses of contaminants, including pesticides, mycotoxins, acrylamide, as well as acute toxicity experiments to validate the safety of using coffee husk, parchment and silverskin as safe ingredients [25]. Similarly, the load of pesticides and other contaminants in wine-making byproducts have been widely studied [26]. The wine-making byproduct extracts used in the present thesis were from commercial grade and were purchased from Natac (product codes N20105891, N20105801 and N20105813 for grape skin, grape seed and grape pomace extracts, respectively). Extracts' food safety sheets included information on microbiological and contaminant quality control analyses.

The next step in the functional food development process involved the selection of the food carrier (Figure 1, Step 4). Yogurt was selected as the most suitable food matrix for the vehiculization of the novel ingredients. This is because yogurt is perceived as the healthiest and most preferred carrier food of functional ingredients by consumers [27,28]. The incorporation of the bioactive ingredients in the food matrix was conducted by a multidisciplinary approach, which is further explained in the following section.

Altogether, findings from the literature review, results previously generated from our research group and those derived from the present investigation allowed us to select coffee and wine-making byproducts as suitable ingredients for the development of sustainable and potentially healthier yogurts.

2. Food applications using coffee and wine-making byproducts

The yogurt formulations developed in the present thesis are summarized in Table 1.

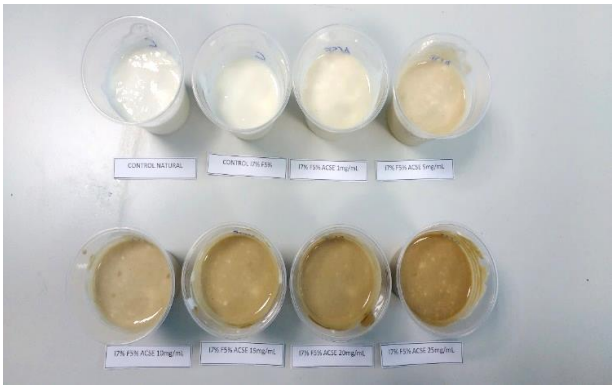
Table 1. Summary of the yogurt formulations used during the present PhD thesis.

	Chapter 2		Chapter 3	Chapter 4
	Study 1	Study 2		
Dietary fiber (g/100 mL)				
FOS	10	-	-	-
Inulin	7	6	6	0, 3, 7, 13
Non-sugar sweetener (g/100 mL)				
Maltitol	-	3	3	-
Byproduct ingredients (mg/mL)				
Grape pomace extract	5	5	5	-
Grape seed extract	5	5	5	-
Grape skin extract	5	5	5	-
Coffee silverskin extract	-	-	4	-
Coffee cascara extract	-	-	10	10

The basal yogurt formulation was first developed adjusting the dose of inulin and FOS. The first prototype included 10 g of FOS and 7 g of inulin, aiming to provide half of the dietary fiber DRI in an 80 g yogurt portion (25 g per day, according to EFSA guidelines). FOS and inulin were included for their role in appetite control and energy intake due to SCFA production from colonic fermentation and their effect on the secretion of gut hormones [29]. FOS was included for its sweetening properties to avoid sugar addition, whereas inulin was included as a texture modifier to improve the yogurts' mouthfeel [30]. The total dietary fiber amount per serving corresponded to 13.6 g, which is within the concentration range of previous studies using inulin and FOS in dairy desserts [31,32]. In addition, daily intakes below 15 g of inulin or FOS had been described to be generally well tolerated [33].

After the obtention of the basal yogurt formulation, the concentrations of the byproduct extracts were optimized. This optimization was assessed considering multiple factors: extract solubility, interaction with compounds from the yogurt matrix, moment of addition of the extracts in the yogurt matrix, product format (set-type yogurt, stirred, liquid), pilot sensory tests, etc. (Figure 4a and b). This optimization resulted in an initial product prototype of yogurts containing wine-making byproducts, inulin and FOS (*Chapter 2, Study 1*) (Figure 4c).

(a)



(b)

(c)

Figure 4. Examples of preliminary experiments on the range of coffee silverskin concentrations in yogurt (a), and on the incorporation of the extract in different yogurt formats (b). First prototype of yogurts containing wine-making byproducts, inulin and FOS (c). From left to right: grape skin, control, grape seed and grape pomace yogurts.

Volunteers in the sensory tastings in Chapter 2, Study 1 complained about the gastrointestinal effects that appeared after the ingestion of the yogurts. This led to the reformulation of the product replacing FOS by the non-sugar sweetener maltitol. FOS was eliminated due to its higher fermentability compared to inulin. Therefore, the following studies used 6% of inulin, which had been described to be well tolerated [34], together with 3% maltitol. In addition, the nutritional trial to assess potential gastrointestinal symptoms derived from inulin consumption was proposed. The optimization of the inulin concentration was designed to determine the dose that could be well tolerated in acute conditions, which would determine how much inulin could be consumed in a single portion and its contribution to the dietary fiber DRI.

This example highlights that product development is an iterative process which involves product reformulation according to the information obtained during the different

experimental procedures. Thus, during the present thesis, product prototypes were improved according to experimental findings (Table 1).

The design of the yogurt formulations containing dietary fiber and the bioactive compounds from the coffee and wine industries was conducted following a multidisciplinary approach that optimizes the technological, health-promoting and sensory properties of the developed products with the same relevance. The analyses conducted in the different chapters of the thesis were summarized attending to these three dimensions in Figure 5. This approach has been recently proposed by Granato *et al*. (2017) [35] for the development of functional foods as a means of promoting market stability and consumer adherence of the launched products. It has been estimated that there is a 70 – 90% risk of market fail within the first two years of launching a new functional product [36], which may be partly attributed to industries focusing on the technical feasibility and overlooking consumers' acceptance, preferences and needs [37].

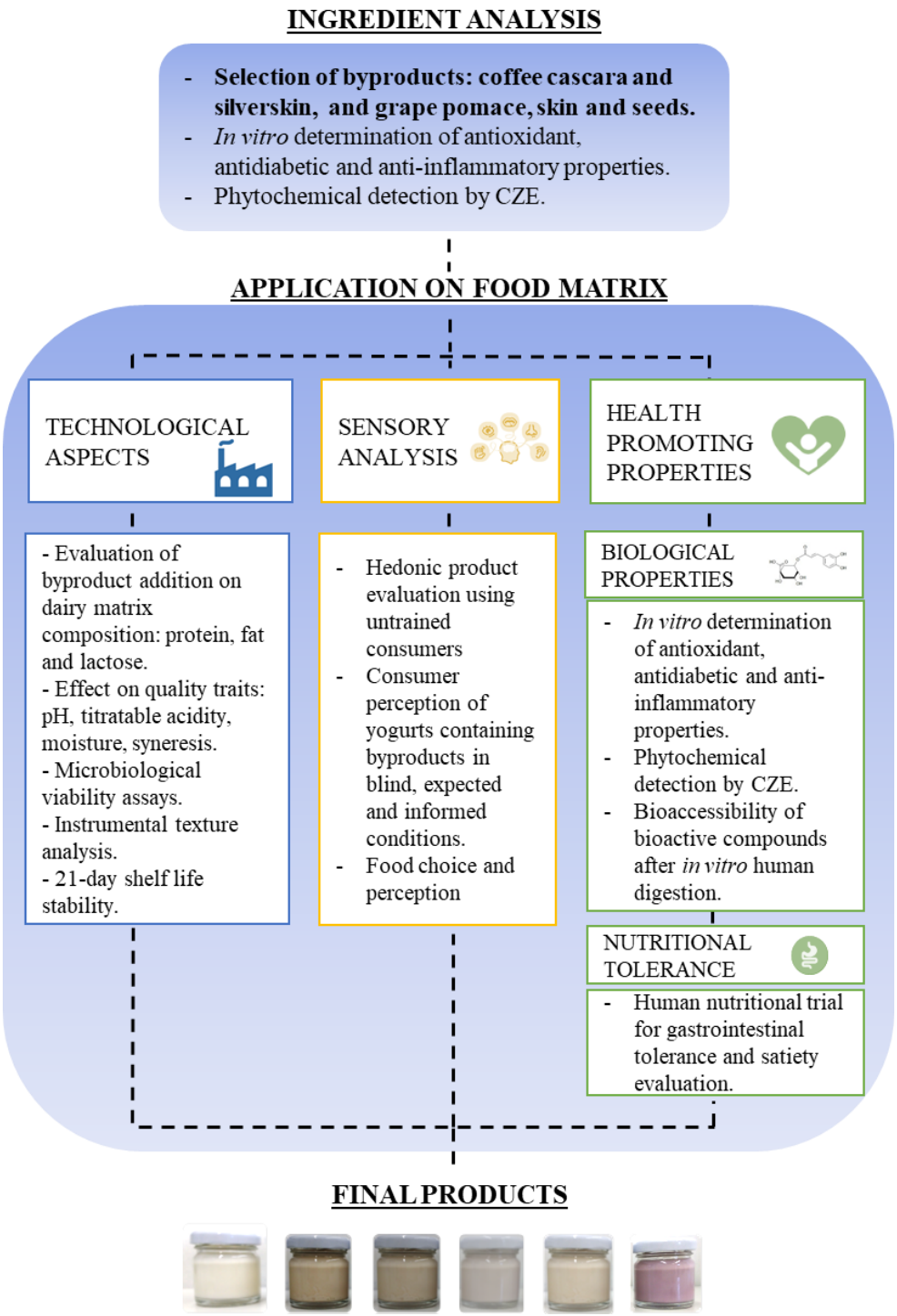


Figure 5. Integrated strategy for the development of the novel yogurts.

2.2. Technological quality of yogurts containing coffee and wine-making byproducts

Byproducts from the coffee and wine industries were successfully incorporated into the yogurt matrix. The study on the shelf life of yogurts containing grape pomace, seeds and skins showed that syneresis during storage was the main issue regarding the physicochemical parameters of the yogurts. Namely, the addition of the wine-making byproducts to the yogurt matrix promoted the liberation of whey after two weeks of cold storage (*Chapter 2, Study 1, Table 4*). Syneresis is considered an important defect by consumers which can lead to product rejection [38]. The increased liberation of whey during storage has also been described in yogurts containing grape pomace flour [39] and green tea powder [40].

It has been well stablished that inulin visibly reduces yogurt syneresis due to its water retention ability [41]. In our study, the control yogurt with dietary fiber showed high syneresis the first day after yogurt manufacturing. This could be explained by the fact that we standardized fermentation time to 5 hours. After this period, yogurts containing wine-making extracts and dietary fiber had already formed a compact curd, whereas the control yogurt showed a less firm curd. This is evidenced by the difference in pH values on day 1 between the yogurt samples (pH = 4.75 in the control, pH range = 4.59 – 4.68 in yogurts containing wine extracts). Therefore, syneresis values in the control yogurt may have been influenced by this procedure. Syneresis rates after the second week of storage were stable, which is in accordance with previous studies [42].

Several authors have explained the increased syneresis rates observed in yogurts containing polyphenol-rich fractions by the protein-polyphenol interaction model proposed by [43]. In this model, the high syneresis rates are attributed to an excess of polyphenols relative to proteins. Polyphenols attach to all the protein binding sites, lowering the changes of finding available protein binding sites to branch two proteins together to form a network, resulting in small aggregates with reduced serum trap in the gel matrix (Figure 6). In our study, the complexity is even greater as it must consider the interactions of the milk proteins, polyphenols from the wine-making extracts, and two types of dietary fiber (FOS and inulin). Therefore, further research is needed to gain insight into the matrix interactions of complex foods.

In relation to the texture parameters in yogurts containing wine-making extracts, inulin and FOS results suggest that there is a slight increasing tendency of the yogurts' firmness and consistency during storage (*Chapter 2, Study 1, Table 4*). This increase may be

explained by the observed postacidification, which causes casein aggregation and further whey release [40].

On the other hand, the incorporation of the coffee cascara extract significantly decreased ($p < 0.05$) the yogurt's firmness and consistency parameters compared to the control (*Chapter 4, Figure 1*). In this sense, the recovery of the yogurt's initial texture parameters when adding increasing concentrations of inulin justified its use as a texturizing agent. In this study, the reduction in yogurt syneresis due to the water retention capacity of inulin was also observed. These results were in agreement with previous studies on yogurt syneresis reduction by inulin [44,45].

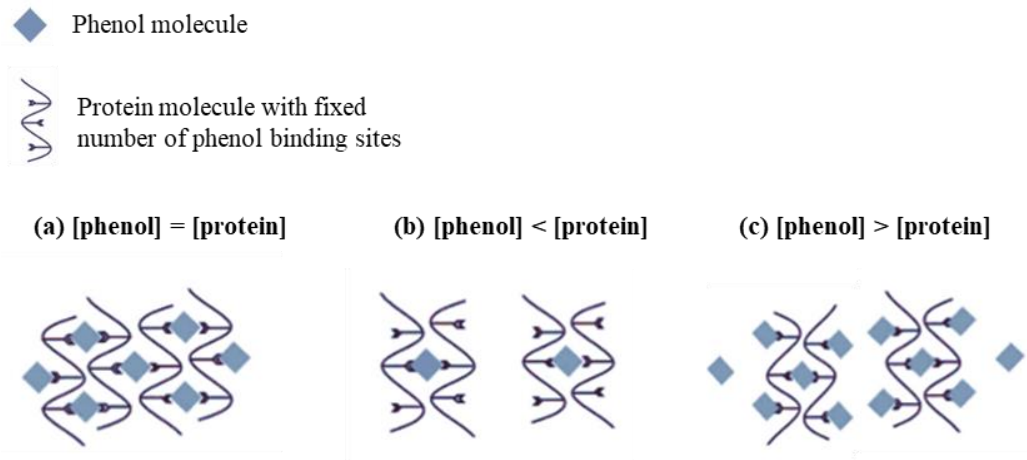


Figure 6. Gel structure stability model of yogurts containing different ratios of protein and polyphenols. Figure adapted from Siebert et al. (1996).

2.2. Sensory analysis and consumer perception

The hedonic tests conducted with the yogurts developed in the present thesis showed high acceptance scores in the control yogurt (7.1), and in yogurts containing coffee cascara (7.0) and grape pomace (6.5), seed (6.2) and skin (6.4) byproducts. As a general rule, a product can be considered as potentially successful from a sensory standpoint when its attributes receive evaluation scores ≥ 6 in a 9-point hedonic scale [35]. In the case of coffee silverskin yogurt, its acceptance was significantly lower (5.1) ($p < 0.01$) than the rest of the formulations (*Chapter 3, Figure 3*). Results on the acceptance of the different sensory attributes (*Chapter 3, Table 3*). suggest that the lower acceptance was

due to the yogurt's taste (4.4), which was significantly lower ($p < 0.01$) than in the other yogurts.

The characteristic flavor of coffee silverskin, as in several polyphenol rich plant extracts, is a potential problem for their use as functional ingredients. Their bitterness and astringency can lead to a decrease in consumer acceptability and willingness to purchase the product [46]. Therefore, alternatives to reduce bitterness and astringency in foods containing polyphenolic extracts have been widely evaluated. In relation to bitterness reduction, various within- (taste-taste) sensory interactions have been proposed for modifying overall flavor perception: chemical interactions which result in altered structures with reduced flavor intensity, oral physiological interactions (interference with taste receptor cells or taste transduction mechanisms associated with another compound), and central cognitive mixture suppression [47,48]. On the other hand, astringency is a tactile sensation caused by the precipitation of salivary proteins, leading to low oral lubrication usually described as dry or rough-mouthfeel [49].

While the addition of sucrose in yogurts containing coffee silverskin extracts may be a successful strategy for reducing bitterness and astringency, this approach is not valid in the development of functional foods for the prevention of diet-related chronic diseases, such as obesity and diabetes (Figure 7). The addition of lower concentrations of the coffee silverskin extract in milk could improve its overall sensory acceptance. However, reducing the concentration of the extract would compromise its biological potential, which did not meet the target antioxidant properties at the current dose (4 mg/mL). Lastly, the concentration of maltitol (3%) could be increased, which is a valid approach for future optimizations of yogurts containing coffee silverksin extract.

Considering the sensory challenge presented by the coffee silverskin extract and the high acceptance scores obtained in the rest of the yogurt formulations, we decided to dismiss coffee silverskin as a candidate for the development of yogurts for chronic disease prevention. Although coffee silverskin application in yogurt was challenging, this extract has been successfully applied in the development of other food products, such as healthy beverages [16], biscuits [17], and it has been used for the isolation of dietary fiber and melanoidins [50].

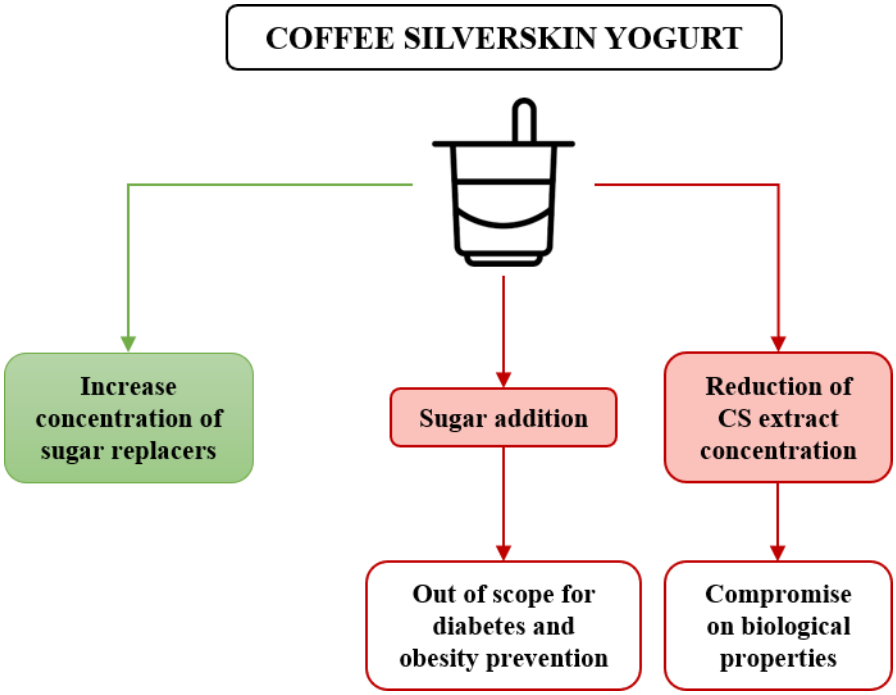


Figure 7. Options for product reformulation for the yogurt containing coffee silverskin extract.

In yogurt formulations containing coffee cascara and wine-making byproducts the combination of inulin and maltitol was more successful. Inulin forms microcrystals when mixed with milk, which forms a fine creamy texture that promotes an oral tactile sensation similar to that produced by fat [21]. Maltitol belongs to the polyol family of "sugar alcohols", which has the advantage of a lower caloric intake than sugars (2.4 kcal vs 4 kcal per gram) and a reduced glycaemic response [51]. These sugar replacers improved the overall acceptance of yogurt formulations containing sustainable ingredients, which makes them valid options as food products positioned for health-conscious consumers.

The study on consumers' perception on the use of wine-making byproducts as ingredients in functional yogurts showed that overall liking of grape pomace, grape seed and grape skin yogurts was influenced by the type of byproduct, the evaluation condition and its interaction. Liking in expected (6.7) and informed (6.6) conditions was significantly ($p < 0.05$) higher than in blind conditions (6.3), suggesting that information regarding the origin of the ingredients did not produce a rejection towards the food products. The byproduct*condition interaction indicates that overall liking depends on the combination of the type of byproduct and the condition stimuli provided. These

interactions provide useful information that can be used towards the marketability of the product. In this sense, the lower expected liking observed in the grape pomace and seed yogurts may be a limiting factor as consumers may not get to purchase the product due to low hedonic expectations of the products. On the contrary, expected liking of grape skin yogurt was very high, which suggest that grape skin may be an appealing ingredient to include in yogurt formulations. In general, results suggest that wine-making byproducts could be well accepted as novel ingredients in dairy foods. Coffee byproduct perception would need to be analyzed separately as the type of byproduct used in yogurt significantly affected liking scores (*Chapter 2, Study 2, Table 1*).

2.3. Biological and nutritional properties of yogurts containing coffee and wine-making byproducts

Yogurts containing bioactive compounds extracted from wine byproducts showed *in vitro* antioxidant capacity (*Chapter 3, Table 1*). The anti-inflammatory properties of the wine-making byproducts were no longer observed once they were incorporated into the yogurt matrix (*Chapter 3, Figure 2B*). The grape skin yogurt showed a significantly higher ($p < 0.01$) inhibition of the activity of α-glucosidase than the control. Therefore, none of the yogurts containing wine-making byproducts showed the target combination of antioxidant, inhibition of the activity of α-glucosidase and anti-inflammatory properties.

On the other hand, yogurts containing coffee cascara and silverskin extracts showed antidiabetic properties (*Chapter 3, Table 1*). To our knowledge, this is the first time that the inhibition potential of the activity of α-glucosidase in yogurts containing coffee cascara and wine-making byproducts extracts has been evaluated. Coffee cascara and silverskin yogurts also showed a significant ($p < 0.05$) anti-inflammatory effect at 10 mg/mL (*Chapter 3, Figure 2B*). However, the antioxidant capacity of coffee silverskin yogurt was similar to the control ($p > 0.05$). The yogurt containing coffee cascara extract was the only formulation that showed *in vitro* antioxidant, antidiabetic and anti-inflammatory potential (Figure 8). Therefore, coffee cascara yogurt was selected the study of the bioaccessibility of its bioactive compounds.

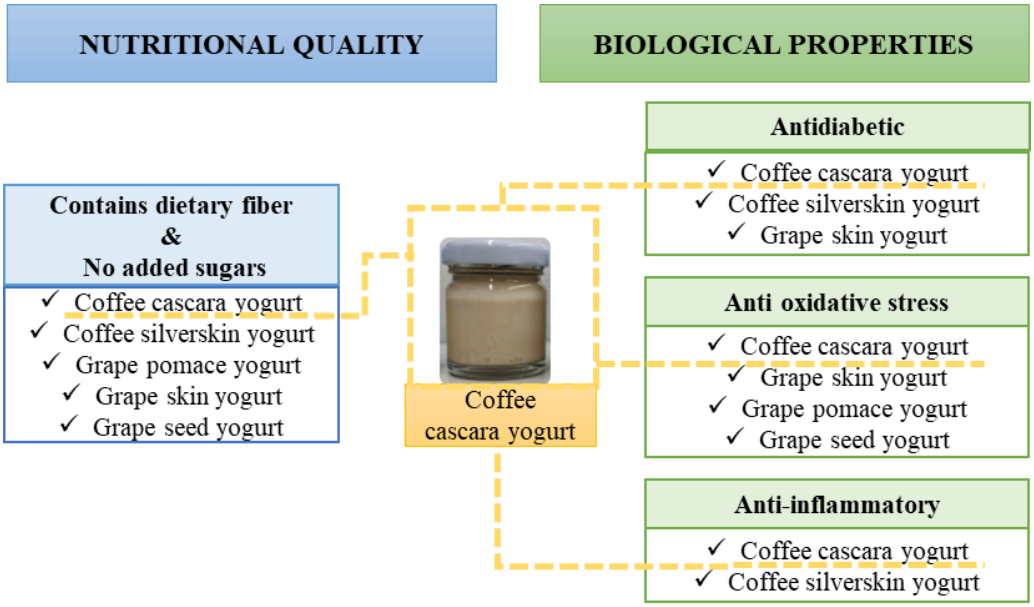


Figure 8. Summary of the nutritional and biological properties observed in the formulated yogurts.

The biological properties of the byproduct extracts were attributed to the bioactive compounds detected by CZE. In this sense, we evaluated their bioaccessibility starting from the moment the byproduct extracts were incorporated in milk for yogurt development until the end of a simulated *in vitro* human digestion process. In the grape pomace and grape skin yogurts, the initially observed bioactive compounds from the respective extracts were still observed in the dairy matrix (C3G and CAT; and C3G in the grape pomace and grape skin yogurts, respectively) (*Chapter 3, Figure 1*). CAT was observed in the grape seed extract but no longer detected in the corresponding yogurt. Similarly, in yogurts containing coffee byproducts only caffeine was observed, although CGA and FA were initially detected in the extracts.

There are two factors which may be potentially affecting the bioaccessibility of bioactive compounds from the byproduct extracts: compound degradation by starter cultures during milk fermentation and a matrix effect. On one hand, some *Lactobacillus* species have been previously described to metabolize plant-derived phenolic compounds, such as hydroxycinnamic acids and tannins [53]. On the other hand, compounds may bind to the food matrix decreasing or losing their efficacy. Binding of polyphenols with protein molecules, driven by hydrogen bonding or hydrophobic interaction, may lead to the

formation of soluble or insoluble polyphenol–protein aggregates [54]. In our study, apart from milk protein-phenol interactions, interactions with inulin need to be considered, as it may be exerting a trapping action on both polyphenols and milk proteins [55].

In our study none of the bioactive compounds were detected after the simulated *in vitro* human digestion, suggesting that the initial compounds were not bioaccessible. However, the cascara yogurt digest showed significantly greater ($p < 0.05$) antioxidant capacity and inhibition of α-glucosidase compared to the control digest. These properties may be attributed to other compounds such as bioactive peptides or other metabolites derived from the cascara extract, as previous studies have suggested that it is the metabolites from the ingested bioactive compounds rather than the original compounds themselves, the ones that exert the biological effects in the body [56]. Further research is needed to elucidate the interactions between the extract polyphenols and inulin in order to gain insight into the bioaccessibility and bioavailability of the byproduct compounds. Current human absorption models of phenolic compounds contained in foods with high dietary content, such as in the developed yogurts, suggest that the major part of antioxidant compounds may be bound to the dietary fiber and reach the colon, where they may be metabolized by colonic microflora [57,58].

Finally, it is relevant to assess the risk-benefit balance under a nutritional perspective in food formulations containing dietary fiber. As stated by the EFSA, “in the risk-benefit assessment, the probability of an adverse health effect or harm (both incidence and severity) as a consequence of exposure should be weighed against the probability of benefit, if both are known to be possible” [59]. In yogurts containing coffee or wine-making byproducts together with inulin the potential risks may be the occurrence of gastrointestinal effects including abdominal discomfort, bloating, flatulence and diarrhea [33]. On the other hand, inulin consumption has been associated with a reduced risk of developing T2D [23,60]. Therefore, results from the balance assessment identified that a concentration of 3% of inulin avoided potential gastrointestinal risks while achieving a yogurt with the nutritional claim “source of fiber” (Figure 9).

Figure 9. Risk-benefit balance assessment of cascara yogurts containing increasing amounts of inulin (0, 3, 7, 13%).

3. Suggested prototype: coffee cascara yogurt

Overall, yogurts containing coffee and wine-making byproducts together with dietary fiber showed promising properties in terms of their technological stability, biological and nutritional quality and organoleptic properties. The coffee silverskin extract might be the most challenging byproduct to use as a food ingredient due to its astringency and bitterness. Based on the results obtained in the present thesis (Figure 10), we propose the coffee cascara yogurt as the most suitable candidate to continue with its validation as a functional food.

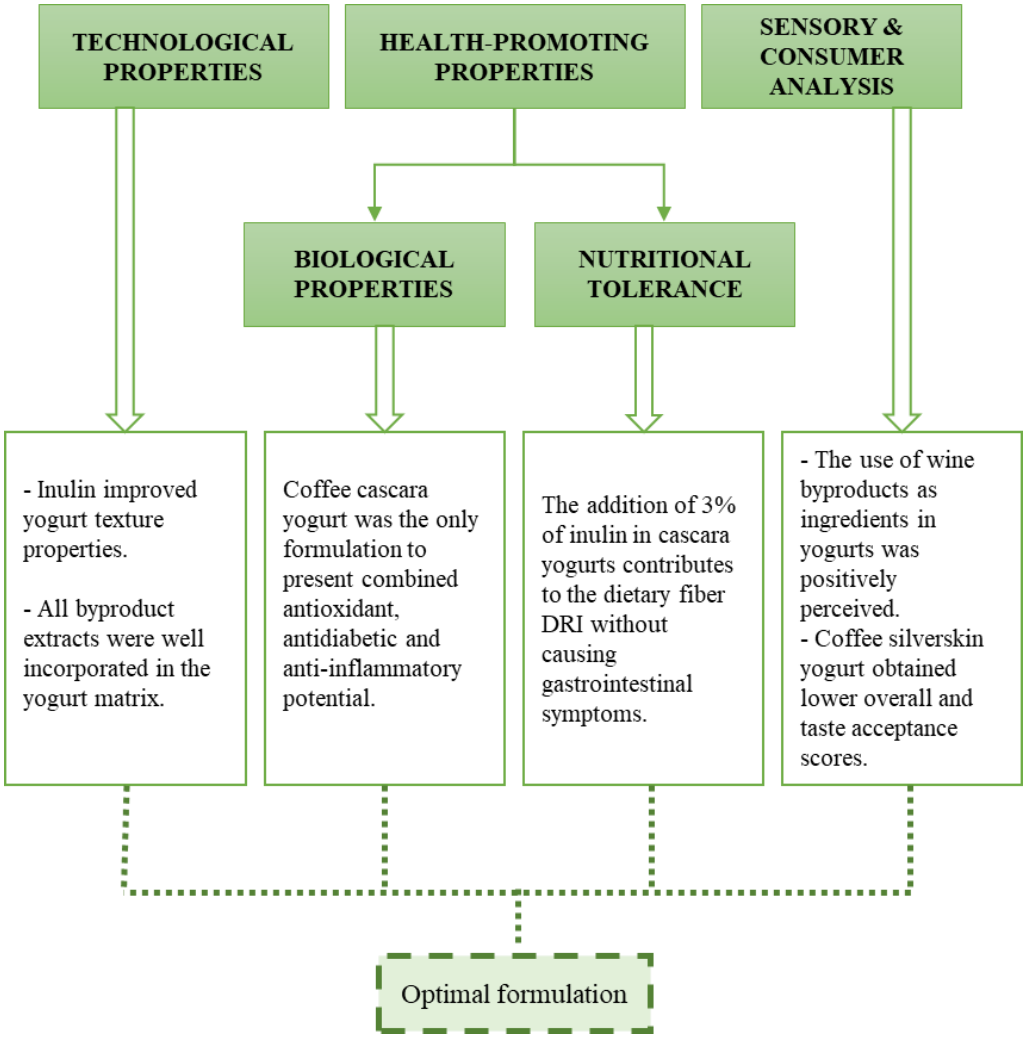


Figure 10. Premises for coffee cascara yogurt selection based on the results obtained for the technological, biological, nutritional and sensory product development analyses.

The combination of 3% inulin and the bioactive compounds from coffee cascara extract with antioxidant, glucoregulatory and anti-inflammatory properties constitutes a sustainable, nutritious and healthy yogurt innovation with high sensory quality (Figure 11). The cascara yogurt achieved the nutritional claims “source of fiber” and “no added sugars”. Information regarding the nutritional and health claims could be stated in the products label to inform the consumer about its properties, which is the last step in the functional food development process (Figure 1, Step 6).

Coffee cascara yogurts stand as an opportunity for using a novel food ingredient, whose utilization is currently demanded by the European industry. The commercialization of coffee cascara yogurts may contribute to attenuate its environmental impact while also contributing to a better dietary environment for diabetic patients. Moreover, the use of byproducts as novel ingredients has the potential to be accepted in the market niche for the healthy and environmentally conscious consumers.

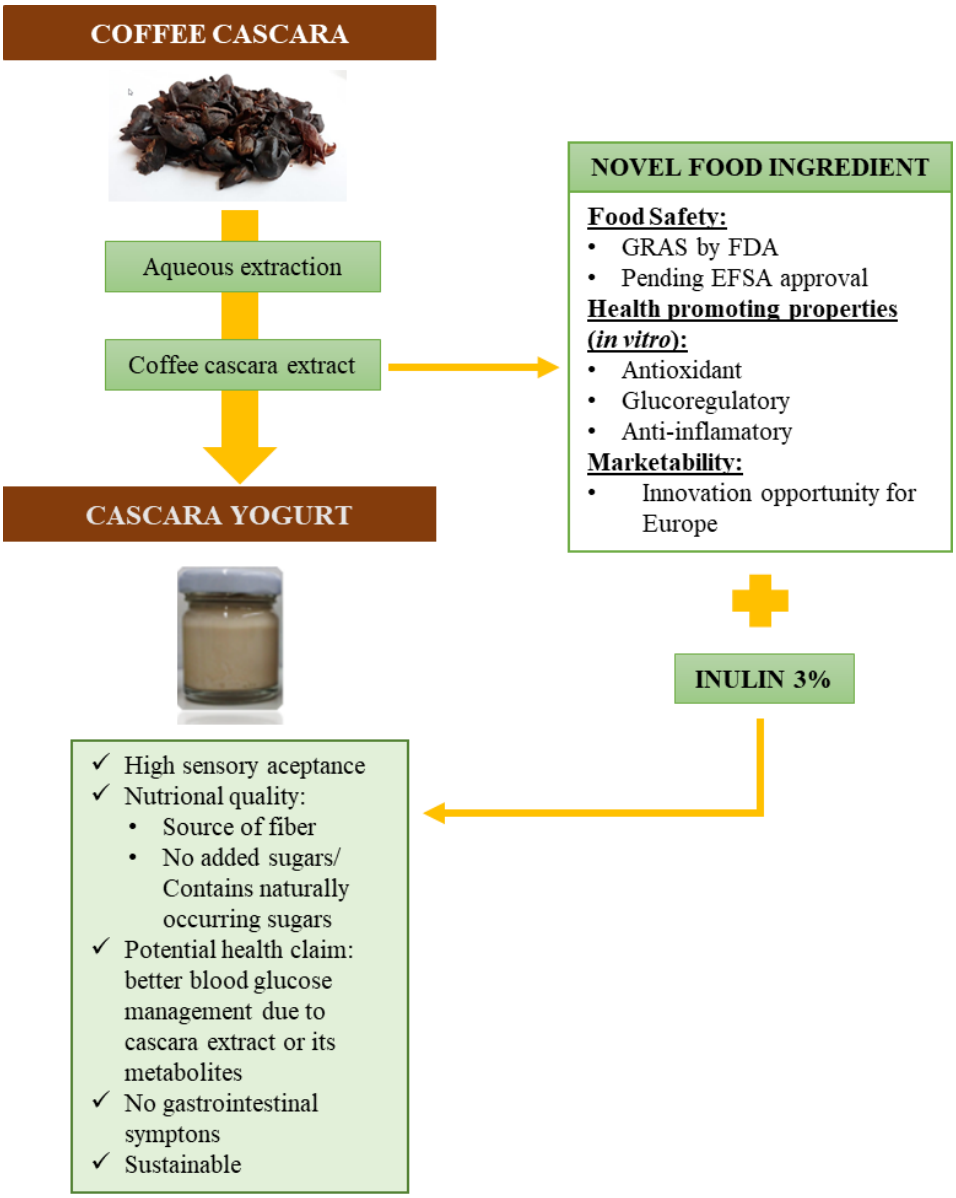


Figure 11. Cascara yogurt product overview.

4. Future perspectives

The development of a functional product necessarily requires the study of its biological properties using *in vivo* experiments in animals and humans (preclinical and clinical trials). Specific biomarkers and proper experimental designs are also required to truly establish whether the wanted biological health effects are being met. Therefore, a double-blind randomized crossover human intervention trial is proposed to continue with the investigation to determine the effect of the intake of the novel cascara yogurt containing inulin in concentrations under 7% in acute and chronic conditions on plasma glucose, plasma antioxidant capacity and appetite-regulating hormones (insulin, GIP, GLP-1, ghrelin). Gastrointestinal tolerance and stool frequency would be assessed to determine whether the yogurt consumption is well tolerated in acute and chronic conditions. In addition, it would be interesting to determine the gastrointestinal tolerance and stool frequency when more than 1 portion of yogurts are consumed during the day, which is in line with the 2 to 3 dairy product servings recommended by SENC (*Introduction, Figure 1*). This would determine whether the yogurts could meet the health claim "maintenance of normal defecation by increasing stool frequency" when a minimum dose of 12 g of inulin is consumed per day. This *in vivo* study would establish whether the sustainable and nutritious yogurts developed in the present thesis can be validated as functional foods (Figure 1, Step 5). However, functional foods are not the sole solution to poor health habits. Therefore, within the scope of a global sustainable health, the development of functional foods should come alongside with consumer education for the promotion of healthy lifestyles and dietary patterns.

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CONCLUSIONS/ CONCLUSIONES

“*It would be so nice if something made sense for a change*”

Lewis Carroll, Alice in Wonderland

CONCLUSIONS

Results derived from the present PhD thesis led to the following conclusions:

1. Plant origin byproducts from the fruit industry have been identified as the group most applied in dairy matrices as food ingredients. Their main function is to act as sources of polyphenols or dietary fiber in the development of yogurt or fermented milk products.
2. Yogurts containing wine-making byproducts (grape pomace, grape seeds and grape skins) are physicochemically stable during a 21-day shelf life period in cold storage.
3. For the first time, consumer perception of the use of wine-making byproducts as sustainable food ingredients in yogurts is evaluated. Information regarding their use increases overall liking of the yogurts and derives in positive concept associations such as “healthy”, “antioxidant”, “high in fiber”, “satiety”, “sustainable” and “novel”.
4. Coffee (cascara and silverskin) and wine (grape pomace, seed and skin) byproduct extracts show *in vitro* antioxidant, antidiabetic and anti-inflammatory properties.
5. Among the developed yogurts containing byproduct extracts at concentrations delimited by its sensory acceptance, only the yogurt containing coffee cascara yogurt presents *in vitro* antioxidant, antidiabetic and anti-inflammatory properties.
6. Coffee silverskin extract added in yogurt at a concentration of 4 mg/mL does not increase the *in vitro* antioxidant properties compared to the control. Moreover, the yogurt presents low overall and taste acceptance scores. Therefore, the coffee silverskin yogurt prototype needs to be reformulated to increase its biological and sensory properties.
7. The bioactive compounds originally detected in coffee cascara yogurt (CGA, FA and CAF) are not bioaccessible after simulated *in vitro* human digestion. However, the coffee cascara yogurt digest presents increased *in vitro* antioxidant capacity and inhibitory action towards the activity of α-glucosidase. Metabolites formed during the digestion process of the foods might be responsible for the observed properties.
8. The concentration of 3% of inulin is the optimal dose for the development of coffee cascara yogurts avoiding potential secondary gastrointestinal symptoms after acute intake of the yogurt.

9. The coffee cascara yogurt containing 3% of inulin is proposed as a nutritious food product, attaining the nutritional claims “source of fiber” and “no added sugars”, with high organoleptic properties. The *in vitro* analysis of its biological properties suggests its potential as a functional food.
10. The use of a multidisciplinary approach in new product development is critical to obtain safe and high quality food products in terms of their physicochemical, sensory and health-promoting properties, to ensure market stability and consumer adhesion to the product once it is launched.

In summary, the present thesis confirmed the feasibility of using coffee and wine-making byproduct extracts as sustainable ingredients in dairy product innovations, providing new knowledge on the effects of adding the byproducts in a yogurt matrix according to its technological, biological and organoleptic properties. The yogurt containing coffee cascara and inulin may stand as a healthier and more sustainable alternative to current market options, whose consumption, together with a balanced dietary pattern, may contribute to the prevention of diet-related chronic diseases such as obesity and T2D.

CONCLUSIONES

Los resultados derivados de la presente tesis doctoral llevaron a las siguientes conclusiones:

1. Los subproductos de origen vegetal de la industria frutícola son el grupo más usado como nuevos ingredientes alimentarios en el desarrollo de productos lácteos. Su función principal es ejercer de fuente de polifenoles o fibra dietética para el desarrollo de yogur y leches fermentadas.
2. Los yogures que contienen subproductos de origen vitivinícola (orujo de uva, semillas de uva y pieles de uva) son fisicoquímicamente estables durante un período de almacenamiento en frío de 21 días.
3. La percepción de los consumidores sobre el uso de subproductos procedentes de la elaboración de vino como ingredientes alimentarios sostenibles en yogures se ha evaluado por primera vez. La información sobre su uso aumenta la aceptación general de los yogures y produce asociaciones positivas con los atributos "saludable", "antioxidante", "alto en fibra", "saciedad", "sostenible" y "novedoso".
4. Los extractos de subproductos de café (cáscara y cascarilla) y vino (orujo de uva, semilla y piel) tienen propiedades antioxidantes, antidiabéticas y antiinflamatorias *in vitro*.
5. El yogurt de cáscara de café es la única formulación que presenta propiedades antioxidanes, antidiabéticas y antiinflamatorias *in vitro*, teniendo en cuenta que la concentración de los extractos en el yogur estuvo delimitada por su aceptación sensorial.
6. La adición de 4 mg/mL de extracto de cascarilla de café en leche no aumenta las propiedades antioxidantes, determinadas *in vitro*, del yogur desarrollado con respecto al yogur control. Además, las puntuaciones de aceptación global y del atributo de sabor del yogur de cascarilla de café desarrollado son bajas, lo que indica que el yogur de cascarilla de café debe ser reformulado para mejorar sus propiedades biológicas y sensoriales.
7. Los compuestos bioactivos detectados originalmente en el yogur de cáscara de café (ácido clorogénico, ácido ferúlic y cafeína) no son bioaccesibles después del proceso de digestión humana simulada *in vitro*. Sin embargo, el digerido del yogur de cáscara de café presenta una mayor capacidad antioxidante *in vitro* y acción inhibitoria hacia la actividad de la α-glucosidasa frente al digerido del control. Los metabolitos formados durante el proceso de simulación de digestión

humana *in vitro* podrían ser responsables de las propiedades biológicas observadas en el digerido del yogur de cáscara.

8. La evaluación de la relación riesgo-beneficio del consumo de yogures que contienen cáscara de café y alto contenido de fibra dietética indica que 3 g de inulina por 100 g de yogur es la concentración óptima para promover la ingesta de fibra dietética en la dieta humana, evitando posibles efectos secundarios gastrointestinales.
9. El yogurt de cáscara de café que contiene 3% de fibra dietética se propone como un producto alimenticio nutritivo, que logra las declaraciones nutricionales "fuente de fibra" y "sin azúcares agregados", con altas propiedades organolépticas. El análisis *in vitro* de sus propiedades biológicas sugiere que tiene un alto potencial como alimento funcional.
10. El uso de un enfoque multidisciplinario en el desarrollo de productos nuevos es fundamental para obtener productos alimenticios seguros y de alta calidad en cuanto a sus propiedades fisicoquímicas, sensoriales y beneficiosas para la salud y para asegurar su estabilidad en el mercado y la adhesión del consumidor al producto.

En resumen, la presente tesis confirma la viabilidad de utilizar extractos de subproductos de café y del vino como ingredientes sostenibles en nuevos productos lácteos y proporciona nuevos conocimientos sobre los efectos de su incorporación sobre las propiedades tecnológicas, biológicas y organolépticas en yogur. El nuevo yogur de cáscara de café e inulina es una alternativa más saludable y sostenible a las opciones actuales del mercado, cuyo consumo, junto con un patrón dietético equilibrado, puede contribuir a la prevención de enfermedades crónicas relacionadas con la dieta, como la obesidad y la diabetes tipo 2.

ANNEX 1: *Vousse*

FOODIO PROGRAM

FOODIO, Food Solutions Master Class, is a multidisciplinary, challenge-based learning program conceived to educate in an international environment, connect business and academia, and stimulate for the creation of new food solutions for future innovation.

This first edition of the program in 2018 connected five academic institutions (the Autonomous University of Madrid and Spanish National Research Council in Spain, Helsinki University in Finland, the Israel Institute of Technology Technion in Israel and the University of Hohenheim in Germany) and three business partner organizations (Valio in Finland, Acesur in Spain and Herbstreith & Fox in Germany).

OBJECTIVE

The goal of this first edition of FOODIO was to develop the healthiest and most appealing dairy product, while contributing to sustainability by using plant-based side-streams from food industry.

PRODUCT DEVELOPMENT

In response to this challenge "Team 1", composed of students from the University of Helsinki, the UAM and Technion, developed "Vousse", a plant-based chocolate mousse. Vousse is protected under an invention disclosure agreement. Therefore, only a general presentation of the product is presented in this Annex.

Vousse was developed as a healthier alternative to current dessert options. It's reduced sugar content and fiber addition allowed our product to have two nutritional claims, "reduced sugar" and "source of fiber". Contribution to sustainability was not only achieved by using the citrus side-streams fibers but also by entering a dairy dessert section with a non-dairy product. This contributed to a significant reduction in the water and carbon footprint of Vousse. However, the main goal was to achieve excellent organoleptic properties. This way, our added value was to provide an excellent sensory experience without contributing to health or environmental deterioration (Figure 1).

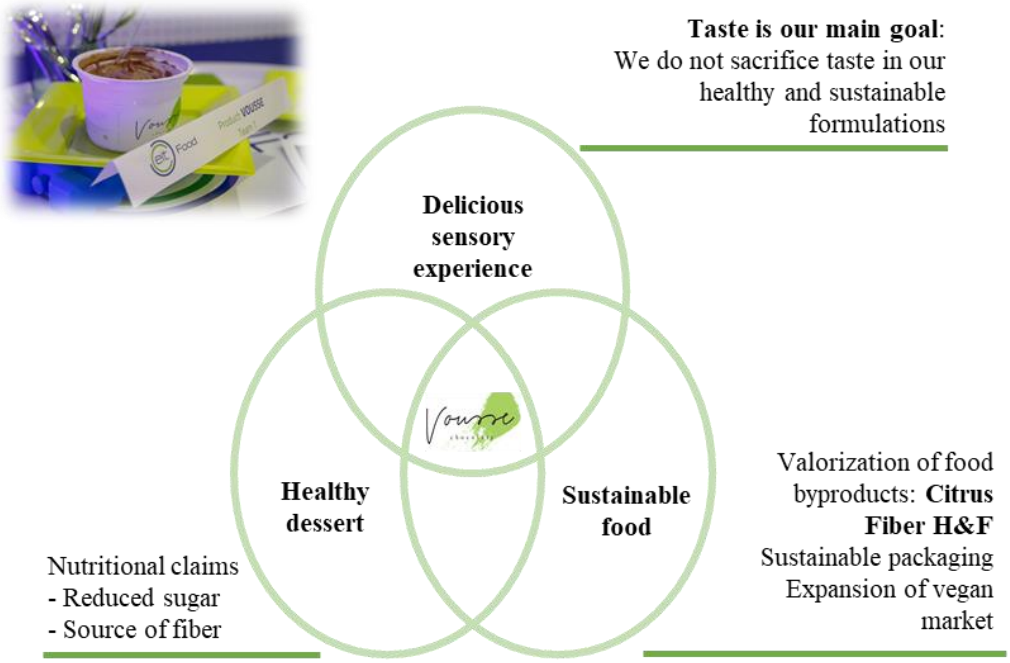

Figure 2. Schematic view of product characteristics of Vousse.

From March to December 2018 our team worked on the business plan and product development for Vousse. The workplan involved:

- Product idea and market analysis.
- Product development tasks: ingredient tests and selection, product physicochemical analyses, quality trait comparison with a competitor chocolate mousse product, nutritional assessment. Trials were conducted in laboratory scale and in an industrial pilot scale in Valio's facilities.
- Sensory analysis in Helsinki (n = 100 consumers).
- Consumer online survey (n = 300 participants) to test the product concept idea acceptability.
- Business plan including financial planning, marketing strategy, company milestones and customer traction.

The responsibilities and participation of M. Iriondo-DeHond involved:

- Team representative and speaker in the preliminary competitive round "3-minute elevator pitch" in the Bootcamp in Helsinki (5 – 8 March 2018).
- Participation in ingredient selection.

- Design, implementation and analyses of the sensory experiments.
- Design, implementation and analyses of the online consumer survey.
- One-week research stay in Helsinki (8 – 12 October 2018) to carry out pilot industry scale test of Vousse in Valio's facilities and conduct the sensory tests.
- Participation in the business plan development: definition of target market and consumers, financial plan and company milestones.
- Team speaker in the presentation of Vousse in the Final Gala in Madrid.

The product was presented in the Final Gala the 12th of December 2018, where Vousse was awarded with the FOODIO EIT FOOD Award to the best product (Figure 2).

Figure 3. Team 1 members awarded with the FOODIO award for the development of Vousse.

ANNEX 2: *Capricho de Vino*

VEGA DE SAN MARTÍN

Vega de San Martín (VSM) is family owned business which produces artisan goat cheese from raw milk. Their business follows a circular economy model: they grow their own crops, which are fed to their own goats (Murciano - Granadina breed), which are milked to produce cheese in their own artisan factory. VSM is the only cheese industry allowed to produce cheese from raw milk with less than 60 days of maturation in the Community of Madrid.

OBJECTIVE

A new product development project was established between VSM and IMIDRA with the following objectives:

- Develop a novel cheese containing wine-making byproducts.
- Identify the ripening time point which maximized the sensory acceptance of the product. Maturation points for conducting analysis were 3, 6 and 9 weeks.

PRODUCT DEVELOPMENT

The participation of M. Iriondo-DeHond in the new product development tests and ripening study involved:

- Study of the alternatives of incorporation of the wine-making byproducts in the different cheese types developed in VSM and selection of the type of cheese which better integrated the new ingredient.
- Selection of the most suitable wine-making byproduct (among grape pomace, grape seeds and grape skins extracts) in the cheese formulation according to its organoleptic properties.
- Evaluation of the physicochemical traits of the new cheese formulation: pH, color, nutrient composition, microbiology viability, instrumental texture profile.
- Sensory profile of the new product: the panel of assessors trained for cheese products from IMIDRA was given specific training for the cheese prototype developed. This training was conducted to use the panel in the ripening study and obtain a sensory description of the new cheese in the different time-points.

- Consumer tests: for the 3 different ripening time-points, overall liking and liking of the “appearance”, “smell”, “taste” and “texture” attributes was assessed using a 9-point hedonic scale. JAR scales were incorporated to obtain information for product reformulation. Also, a preference question was used to rank cheese formulations with different ripening time-points.

CAPRICHO DE VINO

“Capricho de vino” is protected under an invention disclosure agreement. Therefore, only a general presentation of the product is presented in this Annex.

“Capricho de vino” is a cheese made goat raw milk with a minimum ripening time of 3 weeks. It is made with a mixed (enzymic and acid) coagulation. The cheese crust contains the grape pomace extract recovered from the wine-making process.

At a ripening time of 3 weeks, “Capricho de vino” presents a white velvety curd due to the *Penicillium* mold. The curd has a white color, which is characteristic of cheeses made from goat milk. Between the crust and the curd, a thin brown line resembles the addition of the wine extract. The scent has a medium intensity with herbal and fungal notes that are well integrated with the goat component. The texture is firm and buttery to the palate. It has a lactic flavor with elegant acidity, with persistent herbal, fungal and goat aftertaste notes.

“Capricho de vino” was presented in Madrid Fusión in 2017 and it is currently being commercialized by VSM.

ANNEX 3: Yogurts for the prevention of metabolic syndrome

METASIN PROJECT

The main objective of the METASIN project is to develop novel ingredients, foods and multifunctional food supplements capable of influencing the pathologies and risk factors associated with the metabolic syndrome.

OBJECTIVE

The Food Bioscience Group at CIAL-UAM is participating within the METASIN project by collaborating with one of the industrial partners: Feiraco. The main goal is to develop dairy products to potentially reduce the risk of developing metabolic disorders.

PRODUCT DEVELOPMENT

The participation of M. Iriondo-DeHond in the new product development tests included:

- Evaluation of different new functional ingredients.
- Study of alternative technologies for incorporating the new ingredients in the dairy matrix.
- Ingredient selection and optimization of pilot formulations.
- Conducting sensory tests for assessing product acceptance and obtaining information for further improvement by product reformulation.
- Data analysis and presentation of results.

CURRICULUM VITAE

What is on my plate? That is the question that led me to become a scientist. And now, as a scientist, my goal is to bring to that plate healthy and sustainable foods, without losing the sensory pleasure and fun of eating.

Education

- **Doctoral program** in Food Sciences (2015-2019). Universidad Autónoma de Madrid (UAM), Spain.
- **Master's degree** in Bioinformatics (2012- 2013). University of Skövde & Swedish University of Agricultural Sciences, Sweden.
- **Master's degree** in Food Chain Systems (2011- 2012). Cranfield University, UK.
- **Bachelor's degree** in Agriculture Engineering (2006-2012). Universidad Politécnica de Madrid, Spain.

Fellowships and research experience

- **Pre-doctoral Student Fellowship** granted by IMIDRA (January 2015 – January 2019). Fellowship for the development of a doctoral thesis in the Food Science PhD program of Universidad Autónoma de Madrid (UAM), Spain.
- **Master Thesis** (March – July 2013) **"**Role of Chromatin Assembly Factor CAF-1 in establishing DNA methylation in *Arabidopsis thaliana",* University of Skövde & Swedish University of Agricultural Sciences, Sweden. P.I. Prof. Lars Hennig.
- **Master Thesis** (April – September 2012) **"**Development of microarray data analysis pipeline to identify key genes in relation to metabolic spoilage biomarkers in meat", Cranfield University, UK. P.I. Prof. Fady Mohareb.
- **Erasmus scholarship**, Cranfield University, UK (September 2011 – September 2012).
- **UPM Research Fellowship** (September 2013 – December 2013 and June 2011 – July 2011). Research assistant in the study of Transcriptional Networks and their Evolution in the Brassicaceae. Center for Plant Biotechnology and Genomics UPM-INIA. P.I. Prof. Pilar Carbonero.

- **Laboratory technician and field assistant** (July-August, years 2010, 2008, 2007) at Buckler Lab for Maize Genetics and Diversity, Cornell University, USA. Participated in projects: "Dissecting Complex Traits in Maize and Biofuel Grasses by Applying Genomics, Bioinformatics and Genetic Resources" and "Developing Association Mapping in Polyploid Perennial Biofuel Grasses". P.I. Prof. Edward Buckler.

Participation in research projects

- FP16-LACT, IMIDRA. "Elaboración de nuevos productos lácteos funcionales ricos en compuestos antioxidantes" (2016 - 2018). Participation: principal investigator.
- SUSCOFFEE, AGL2014-57239-R, MINECO. "Producción y consumo sostenibles del café: validación de subproductos como ingredientes alimentarios" (2015 - 2018). Participation: collaborator from CIAL-CSIC.
- METASIN, CIEN, CDTI. "Investigación, desarrollo e innovación en nuevos alimentos multifuncionales para síndrome metabólico" (2015 – 2018). Participation: collaborator from CIAL-CSIC.
- SYMBIOSIS-EU, EU-FP7 project 211638, "Scientific synergism of nano-bio-info-cogni science for an integrated system to monitor meat quality and safety during production, storage, and distribution in EU" (2008 - 2012). Participation: collaborator from Cranfield University.

Publications

Publications derived from PhD Thesis: (see "*List of publications*" section for publication details).

- Research articles: published (2), submitted (4), in preparation (2).
- Book chapters: published (2).
- Participation in conferences: 16 (6 oral communications and 10 posters).

Research articles previous to PhD thesis:

— Mohareb, F., **Iriondo, M**., Doulgeraki, A., van Hoek, A., Aarts, H., Bessant, C. and Nychas, G., (2015), "Identification of meat spoilage gene biomarkers in Pseudomonas putida using gene profiling", *Food Control*, 57, pp.152-160.

— Iglesias-Fernández, R., Wozny, D., **Iriondo-de Hond, M**., Oñate-Sánchez, L., Carbonero, P., and Barrero-Sicilia, C., (2014), "The AtCathB3 gene, enconding a cathepsin b-like protease, is expressed during germination of Arabidopsis

thaliana and transcriptionally repressed by the bZIP protein GBF1", *Journal of Experimental Botany*, doi: 10.1093/jxb/eru055.

Merits and awards

- Best communication awards:
  - **Elsevier Poster Award** for the conference communication "Application of coffee and wine byproduct extracts as novel ingredients in sustainable functional yogurts" 5th International ISEKI food Conference, Stuttgart, Germany, 3-5th July 2018.
  - **Second prize in the category of Food Science and Innovation** for the conference communication "Determinación in vitro del potencial antioxidante y respuesta de glucosa postprandial de yogures formulados con subproductos de la industria del café", XXII Jornadas de Nutrición Práctica y XII Congreso Internacional de Nutrición, Alimentación y Dietética, 11-12th April 2018, Madrid, Spain.

- International program awards:
  - **FOODIO EIT-FOOD Award** for the development of "Vousse", a sustainable and healthy plant-based product using byproducts from the juice industry. Prize sponsored by Valio, 12th December 2018, Madrid, Spain.
  - **Exceptional Student Prize** for MSc in Food Chain Systems, 2012. Prize sponsored by Tillery Valley.

Professional affiliations and membership

- (2018 – Present) European Sensory Science Society (E3S) Student Subdelegate in Spain.
- (2017 – Present) AlimentUS, Spain. Cofounder and president of association.
- (2014 – Present) Amigos del Patrimonio Gastronómico Español (APGE), Spain. Cofounder and vicepresident.
- (2015 – Present) Institute of Food Technologists (IFT), USA. Member.

PhD TRAINING ACTIVITIES

Participation in scientific conferences

1. **Iriondo-DeHond, M**., Iriondo-DeHond, A., Herrera, T., Sorzano, C.O., Miguel, E., del Castillo, M.D., "Randomized crossover nutritional trial of yogurts containing dietary-fiber and coffee cascara extract in healthy adults", IV World Congress of Public Health Nutrition and XII Congreso de la Sociedad Española de Nutrición Comunitaria (SENC) NUTRIMAD 2018, 24-27th October 2018, Madrid, Spain. Poster.
2. Cruz Maceín, J.L., **Iriondo-DeHond, M**., Miguel, E., "Salud o sabor: el consumo de queso en la Comunidad de Madrid", III Congreso Español de Sociología de la Alimentación, 27-28th September 2018, Oviedo, Spain. Oral presentation.
3. **Iriondo-De Hond, M**., Martín, A., Miguel, E., Del Castillo, M.D. "Application of coffee and wine byproduct extracts as novel ingredients in sustainable functional yogurts" 5th International ISEKI food Conference, Stuttgart, Germany, 3-5th July 2018. Poster.
4. **Iriondo-De Hond, M**., Martín, A., Miguel, E., Del Castillo, M.D. "Determinación in vitro del potencial antioxidante y respuesta de glucosa postprandial de yogures formulados con subproductos de la industria del café", XXII Jornadas de Nutrición Práctica y XII Congreso Internacional de Nutrición, Alimentación y Dietética, 11-12th April 2018, Madrid, Spain. Oral presentation.
5. **Iriondo-De Hond, M.,** Blázquez, J.M., Guzmán, M., Iriondo-DeHond, A., del Castillo, M. D., Miguel, E., "Sustainable functional yogurts for chronic disease prevention", 31st EFFoST International Conference, 13-16th November 2017, Barcelona, Spain. Poster.
6. Miguel, E., Antón-Rodríguez, J., Álvarez-Teno, A., **Iriondo-de Hond, M**., "Clasificación de quesos de oveja y cabra de la Comunidad de Madrid mediante análisis descriptivo cuantitativo", II Congreso de la Asociación Española de Profesionales del Análisis Sensorial, 18-20th October 2017, Valencia, Spain. Poster.
7. Miguel, E., Antón-Rodríguez, J., Álvarez-Teno, A., **Iriondo-de Hond, M**., "Selección de variables sensoriales útiles para diferenciar los quesos de oveja y cabra que se consumen en Madrid en función del tiempo de maduración, tipo de

leche o de elaboración", II Congreso de la Asociación Española de Profesionales del Análisis Sensorial, 18-20th October 2017, Valencia, Spain. Oral presentation.

8. del Castillo, M.D., Iriondo-DeHond, A., Martinez-Saez, N., Fernández-Gómez, B., **Iriondo-DeHond, M**., Velazquez-Escobar, F., Zhou, J.R., "Applications of recovered compounds in food products", Fourth International Conference on Cocoa Coffee and Tea, 25-28th June 2017, Turin, Italy. Oral presentation.
9. **Iriondo-DeHond, M**., Iriondo-DeHond, A., Fernández-Gómez, B., Miguel, E., del Castillo, M.D., "Sensory optimization of functional yogurts containing coffee byproduct ingredients", Fourth International Conference on Cocoa Coffee and Tea, 25-28th June 2017, Turin, Italy. Poster.
10. **Iriondo-DeHond, M**., Álvarez-Teno, A., del Castillo, M.D., Miguel, E., "Efecto de la adición de subproductos vitivinícolas como nuevos ingredientes en las características fisicoquímicas y sensoriales del queso", IX Congreso CyTA-CESIA, 16-18th May 2017, Madrid, Spain. Poster.
11. **Iriondo-De Hond, M**., Iriondo-DeHond, A., Fernández-Gómez, B., Miguel, E., del Castillo, M.D., "Optimización de la formulación y la calidad sensorial de nuevos lácteos funcionales", II Jornadas Científicas CIAL Forum, 16-17th November 2016, Madrid, Spain. Poster with oral presentation.
12. **Iriondo-De Hond, M**., Miguel, E., del Castillo, M.D., "Selección de subproductos vitivinícolas como ingredientes para lácteos funcionales", Segundo Congreso Iberoamericano de Ingeniería de los Alimentos CIIAL 2016, 13-14th November 2016, Montevideo, Uruguay. Poster.
13. **Iriondo-DeHond, M**., Antón-Rodríguez, J., Álvarez-Teno, A., Miguel, E., "Efecto de la adición de vino durante diferentes etapas del proceso de elaboración, en la calidad fisico-química y sensorial del queso de cabra", II Jornada de Ciencia y Gastronomía, 17-18th October 2016, Madrid, Spain. Poster with oral presentation.
14. Miguel, E., Álvarez-Teno, A., **Iriondo-DeHond, M**., Mancho, C., "Correlaciones entre el contenido de ácidos grasos totales y el perfil olfativo de quesos de oveja y cabra", XVII Congreso Internacional SEOC 2016, 14-16th September, Talavera de la Reina, Spain. Poster.
15. Miguel, E., **Iriondo-DeHond, M**., Álvarez-Teno, A., Mancho, C., "Parámetros de textura y del perfil olfato-gustativo importantes para la impresión global de los quesos", XVII Congreso Internacional SEOC 2016, 14-16th September, Talavera de la Reina, Spain. Poster.
16. **Iriondo-DeHond, M**., Álvarez-Teno, A., Mancho, C., Miguel, E., "Relationship between sensory texture and flavor variables with overall cheese

impression", Eurosense 2016 Conference, 11-14th September, Dijon, France. Poster.

Prizes and awards

- **FOODIO EIT-FOOD Award** for the development of "Vousse", a sustainable and healthy plant-based product using byproducts from the juice industry. Prize sponsored by Valio, 12th December 2018, Madrid, Spain.
- **Elsevier Poster Award** for the conference communication "Application of coffee and wine byproduct extracts as novel ingredients in sustainable functional yogurts" 5th International ISEKI food Conference, Stuttgart, Germany, 3-5th July 2018.
- **Second prize in the category of Food Science and Innovation** for the conference communication "Determinación in vitro del potencial antioxidante y respuesta de glucosa postprandial de yogures formulados con subproductos de la industria del café", XXII Jornadas de Nutrición Práctica y XII Congreso Internacional de Nutrición, Alimentación y Dietética, 11-12th April 2018, Madrid, Spain.

International programs

Participation in the FOODIO program by EIT FOOD (March – December 2018). The educational program consisted in the development of a sustainable and healthy dairy food using byproducts from the juice industry as ingredients. M. Iriondo-DeHond worked together with students from Finland and Israel to develop "Vousse" (Annex 2). The following activities were performed as part of the program:

- Two research stays in the University of Helsinki, Finland, to work on the product development.
- Ten-month involvement in the product development, business plan, sensory and consumer analyses.
- Speaker in the first product classification round in the "Elevator Pitch" competition.
- Speaker in the Final Gala product presentation.

Supervision of undergraduate research

2018 - Co-supervisor of the final degree project of Alba Martínez Ginel, "Desarrollo de yogur funcional a base de subproductos de café de elevada calidad sensorial", Universidad Politécnica de Madrid, Spain.

2017 - Co-supervisor of the final degree project of José Manuel Blázquez Duff, "Elaboración y caracterización de nuevos productos lácteos funcionales ricos en compuestos antioxidantes", Universidad de Alcalá, Spain.

Training courses

1. Course: "Estudio de la respuesta de los consumidores", 27-28th September 2018, IATA-CSIC, Valencia, Spain.
2. Conference: "I International Meeting of European Sensory Science Students (E3S)", Asociación Española de Profesionales del Análisis Sensorial (AEPAS), 22nd November 2017, Madrid, Spain.
3. Course: "Application of rapid sensory methods to product development and optimization", 23-25th October 2017, IATA-CSIC, Valencia, Spain.
4. Course: "Estilos de vida saludables", 10-11th July 2017, XVII Escuela de Nutrición Francisco Grande Covián, Santander, Spain.
5. Conference: "Día Mundial de la Leche", 1st June 2017, Federación Nacional de Industrias Lácteas, Madrid, Spain.
6. Seminar: "Zumo de fruta: procesos de elaboración y legislación", 19th April 2017, CIAL-CSIC, Madrid, Spain.
7. Course: "Manejo de texturometría avanzada", 28th February 2017, ANAME, Madrid, Spain.
8. Seminar: "Clasificación quimiométrica aplicada a agroalimentos", 31st Janurary- 1st February 2017, CIAL-CSIC, Madrid, Spain.
9. Seminar: "Microbiota intestinal y empleo de lácteos en la alimentación", Universidad Complutense de Madrid- Instituto Danone, 25th November 2016, Madrid, Spain.
10. Seminar: "Nutrición personalizada ¿reto o realidad?, Instituto de Investigación en Ciencias de la Alimentación, CIAL-CSIC, 16th November 2016, Madrid, Spain.

11. Seminar: "Nuevas estrategias en alimentación y nutrición humana", Real Academia Nacional de Medicina- Instituto Danone, 20th October, 2016 Madrid, Spain.
12. Online course: "Food Texture Fundamentals" Institute of Food Technologists, USA, October 2016.
13. Course: "Afinado de quesos, defectos y alteraciones", 2-3rd June 2016, Instituto Tecnológico Leartiker, Markina-Xemein, Spain.
14. Course: "Diseños factoriales y análisis de experimentos en ciencias naturales", Museo Nacional de Ciencias Naturales-CSIC, 15-26th February 2016, Madrid, Spain.

Scientific transfer activities

1. Participation in newspaper interview "Los alimentos hechos con residuos que sí te comerías", ABC, January 2019. Available at: https://www.abc.es/familia/vida-sana/abci-alimentos-hechos-residuos-si-comerias-201901040235_noticia.html
2. Participation in the radio program Capital Madrid, with Mª Jesús Latasa, to present the awarded Foodio food product "Vousse", 19th Dcember 2018, Madrid, Spain.
3. Participation in the radio program "Hoy por hoy Madrid", Cadena Ser, with Guillermo Reglero, in the session about The Future of Foods, 27th November 2018, Madrid, Spain.
4. Participation in the conference "Reflexiones de Innovación", the official presentation of EIT FOOD CLC SOUTH, 11th July 2018, Madrid, Spain.
5. Secretary and speaker in the course: "Descubre el queso: denominaciones de origen y análisis sensorial", Curso de Verano de la URJC, 29th June 2018, Madrid, Spain.
6. Participation in the "Thesis in 3 minutes" program, 26th April 2018, UAM, Madrid.
7. Participation in the television program "Comando Actualidad", RTVE, program focused about the amount of sugar contained in foods, CIAL-CSIC, 15th February 2017.
8. Presentation of the cheese "Capricho de Vino" developed as part of the thesis of M. Iriondo-DeHond in Madrid Fusión, 25th January 2017, Madrid, Spain.
9. Participation in the television program "De Origen Madrid", Telemadrid, showing the research carried out in the field of cheese by IMIDRA, 18th January 2017, Madrid, Spain.

10. "Introducción al análisis sensorial de alimentos. Cata de productos lácteos". Practical course taught by E. Miguel and **M. Iriondo-DeHond** as part of the XVI Semana de la Ciencia, 8-11th November 2016, Alcalá de Henares, Spain.
11. "Textura de los alimentos. Funcionamiento de un texturómetro, escalas texturales. Modificación de texturas en una receta", Practical course taught by E. Miguel, B. Beltrán and **M. Iriondo-DeHond** as part of the conference program II Jornada de Ciencia y Gastronomía, 17-18th October 2016, Madrid, Spain.
12. "Oportunidades de innovación dentro de la industria láctea". Seminar taught by **M. Iriondo-DeHond** as part of the course: "Oportunidades y normativa de la innovación en el sector agrario e industria asociada", Cursos de Transferencia al Sector Agrario-IMIDRA. 5-9th September 2016, Madrid, Spain.

Social entrepreneurship

(2017- present) Co-founder and president of the non-profit association "AlimentUS", aiming to transfer scientific knowledge in Food Science and Nutrition to the general public. The association was founded by students from the Bioscience research group (CIAL-CSIC) and IMIDRA.

“*IN THE END… We only regret the chances we didn’t take,*

the relationships we were afraid to have,
and the decisions we waited too long to make.”

Lewis Carroll