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Optimisation of a blend of emulsifier substitutes for clean-label artisanal ice cream

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ABSTRACT

The present work aims at the optimisation of a blend of natural ingredients (i.e., citrus fibres - CF, α-cyclodextrin - ACD, and whey protein concentrate - WPP) to be used as substitute of mono- and di-glycerides of fatty acids (MDG) in clean-label ice cream. According to a I-Optimal mixture design, fourteen formulations were produced and characterized (ice cream mix density, soluble solid content, rheological behaviour; ice cream overrun, firmness, melting behaviour, colour); as a comparison, a reference ice cream (REF) containing MDG was also prepared and tested. Applying the Response Surface Methodology, significant models ($p \leq 0.01$), mainly linear and quadratic, were calculated for all the response variables and then used for the optimisation of the substitute blend, with the following targets: maximising overrun; obtaining firmness, initial time of melting, and melting rate in the ranges 10–20 N, 15–18 min, and 2–3 g/min, respectively. The best solution (desirability = 0.713) resulted in an emulsifier mixture composed of 1.1 g/100 g ACD, 1.75 g/100 g WPP, and 0.16 g/100 g CF. The use of such a mixture in ice cream gave a product with characteristics similar to REF or even better, thus demonstrating the possibility to produce a high-quality clean-label ice cream.

1. Introduction

The increasing demand by industry and consumers for new and healthier food products requires constant research of alternative ingredients. The lifestyle changes have increased the consumers' awareness that specific food components are highly related to the increased risk of developing a disease (e.g., type II diabetes, obesity, cardiovascular diseases) (Caterson et al., 2004). However, finding a healthy, environmentally friendly ingredient and at the same time developing a tasty and successful food product is a real challenge. Consumers are more and more suspicious about food additives that are indicated on the labels by the code E of the European law (Reg. (EC) No 1333/2008); they tend to misinterpret and consider the coded additives as unsafe and/or artificial. For this reason, industries and researchers have focused their attention on the development of clean-label foods with quality properties comparable to those of the conventional products containing the E-coded food additives or even better. The clean-label ingredients can derive from both vegetable (e.g., plant proteins or fibres) (Ho et al., 2021) and animal (e.g., whey proteins) (Levin et al., 2016) sources and are intended for a wide array of food products, ice cream included. In a previous study, different E-free emulsifier substitutes were tested in artisanal ice cream, evaluating their effects on the quality of both mixes and final products in comparison to the commonly used mono- and di-glycerides of fatty acids (MDG), which are coded as E471 (Loffredi et al., 2021). However, no one of the tested ingredients was as effective as MDG on ice cream properties, thus suggesting that an optimised combination of the different substitutes can be a valuable strategy to produce ice cream with good quality and clean label.

The best combination of different ingredients cannot be assessed without performing many laboratory tests highly demanding in terms of materials, resources, and time. Useful tools to overcome these issues are the Design of Experiments (DoE) and Response Surface Methodology (RSM), which are increasingly gaining attention not only in academic research but also in the industrial context (Granato & de Araújo Calado, 2014). For the optimisation of formulations, the mixture designs are a good option, allowing the determination of the optimal ingredient blend (mixture) composition (Davidov-Pardo et al., 2013; Gava et al., 2020; Granato & de Araújo Calado, 2014; Maciel et al., 2020). The application of mixture designs is found in several areas, from construction materials (Attah et al., 2020), bioenergy (Wongarmat et al., 2021), and dying industry (Heryanto et al., 2020), to the food sector (Squeo et al., 2021). In the field of ice cream, BahramParvar et al. (2015) applied a

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simplex-centroid mixture DoE to the optimisation of a stabilizer blend for ice cream manufacture. They studied three natural stabilizers (i.e., basil seed gum, carboxymethyl cellulose, and guar gum) and two possible blend concentrations in the ice cream (0.15 and 0.35%), producing twenty ice cream formulations. From the obtained RSM models, the authors optimised the stabilizer blend (84.43% basil seed gum and 15.57% guar gum) and the concentration of use (0.15%) by maximising mix viscosity and ice cream overrun, while minimising extrusion temperature and melting rate.

Considering the difficulties in identifying the ideal MDG substitute for ice creams and the power of DoE methodology, the present work aims at the optimisation, by the I-Optimal mixture design coupled with RSM, of a blend of natural ingredients (i.e., citrus fibres, α-cyclodextrin, and whey protein concentrate) to be used as emulsifier alternative in high-quality clean-label ice cream formulations. The blend ingredients and their concentration ranges were chosen based on the results of previous works (Loffredi, 2018; Loffredi et al., 2021). Among the different types of mixture design, the I-Optimal was chosen because it focuses on maximising the predictive accuracy, thus providing the possibility to predict the optimal concentrations of the blend ingredients in specific conditions within the defined experimental range (Heryanto et al., 2020).

2. Materials and methods

2.1. Ice cream ingredients and formulations

Ice cream mixes were produced using 75.2 g/100 g pasteurised whole milk (Granarolo Alta Qualità, Granarolo S.p.A., Milan, Italy), 3.2 g/100 g pasteurised cream (Latte Milano, Latteria Soresina S.c.a., Soresina, Italy), 3.5 g/100 g skim milk powder, 12.0 g/100 g sucrose, 3.0 g/100 g dextrose, and 0.13 g/100 g stabilizers (guar gum and locust bean gum). All the powdered ingredients were kindly provided by Comprital S.p.A. (Settala, Italy). The remaining 3.0 g/100 g were covered by a mixture of emulsifier substitutes, according to the DoE reported in Section 2.2. The considered emulsifier substitutes were as follows: whey protein concentrate with high phospholipid content (Lipamine M 20, Lecico GmbH, Hamburg, Germany; phospholipid content 16–21 g/100 g; code WPP), α-cyclodextrin (Cavamax W6 Food, Wacker Chemie AG, Munich, Germany; code ACD), and citrus fibres (Herbacel AQ Plus Citrus N, Herbafood Ingredients GmbH, Werder, Germany; code CF).

Besides the DoE samples, a reference ice cream mix containing 0.3 g/100 g MDG (Comprital S.p.A., Settala, Italy) as emulsifiers was produced in duplicate with the following formulation: 77.0 g/100 g pasteurised whole milk, 3.1 g/100 g pasteurised cream, 4.5 g/100 g skim milk powder, 12.0 g/100 g sucrose, 3.0 g/100 g dextrose, and 0.13 g/100 g stabilizers.

All the ice cream formulations were balanced in terms of fat (4.2 ± 0.2 g/100 g), sugar (20.9 ± 0.2 g/100 g), and total solid (31.6 ± 0.4 g/100 g) content.

2.2. Experimental design

An I-Optimal Mixture Design (Design Expert, v.10.0.03, Stat-Ease Inc., Minneapolis, MN, USA) was applied to evaluate the overall effect of the three tested emulsifier substitutes and to optimise the blend composition. The chosen design allows the evaluation of experimental points not equally weighted. Indeed, in a previous work (Loffredi et al., 2021), an excessive and detrimental increase of ice cream mix apparent viscosity was observed for high dosage of CF. Thus, the sum of the three ingredients (WPP, ACD, and CF) was set to 3 g/100 g of the ice cream mix, with a constraint of a maximum of 1 g/100 g for CF. The experimental design generated fourteen formulations, including the repetition of four recipes for the estimation of the internal error (Table 1). Samples were all produced in a randomised order to avoid possible systematic errors and to minimise the effects of unexpected variability.

Table 1
Actual and pseudo factor levels of α-cyclodextrin (ACD), whey protein concentrate with high phospholipid content (WPP), and citrus fibre (CF) in the ice cream formulations of the I-Optimal mixture experimental design developed for the substitution of mono- and di-glycerides of fatty acids in artisanal ice cream.

Sample*	Actual levels (g/100 g)			Pseudo levels		
	ACD	WPP	CF	ACD	WPP	CF
1	3.00	0.00	0.00	1.000	0.000	0.000
2a	1.50	1.50	0.00	0.500	0.500	0.000
2b	1.50	1.50	0.00	0.500	0.500	0.000
3	0.00	3.00	0.00	0.000	1.000	0.000
4	2.12	0.63	0.25	0.708	0.208	0.083
5	0.63	2.12	0.25	0.208	0.708	0.083
6a	1.25	1.25	0.50	0.417	0.417	0.167
6b	1.25	1.25	0.50	0.417	0.417	0.167
7	0.00	2.50	0.50	0.000	0.833	0.167
8a	2.00	0.00	1.00	0.667	0.000	0.333
8b	2.00	0.00	1.00	0.667	0.000	0.333
9a	1.00	1.00	1.00	0.333	0.333	0.333
9b	1.00	1.00	1.00	0.333	0.333	0.333
10	0.00	2.00	1.00	0.000	0.667	0.333

*a, b stands for production replicates.

2.3. Ice cream production

The ice cream production followed the method described by Moriano and Alamprese (2017a). By using a Pastomaster 60 Tronic (Carpigiani S.r.l., Anzola Emilia, Italy), mix batches (15 kg each) were produced and pasteurised for 1 min at 85 °C. After the aging step at 4 °C for 24 h, a Labotronic 20–30 batch freezer (Carpigiani S.r.l, Anzola Emilia, Italy) was used to freeze and whip mix aliquots (3 L each). Time and temperature of ice cream extrusion were recorded by using a chronometer and a spirit thermometer. Immediately after extrusion, ice cream samples were packaged and stored at −30 °C for 24 h. A conditioning step at −16 °C for 24 h was then carried out before analyses.

2.4. Ice cream mix analyses

The ice cream mix analyses, described in detail by Moriano and Alamprese (2017a), included the evaluation of density (g/mL; n = 3), soluble solid content (°Bx; n = 5), and rheological behaviour (n = 3). The latter was performed by measuring the mix flow curves at 4 °C in the 20-500 s^{-1} range of shear rate, by using a Physica MCR 102 rheometer (Anton Paar, Graz, Austria) equipped with coaxial cylinders (CC27) and managed by the RheoCompass software (v. 1.21.652, Anton Paar, Graz, Austria). The results are presented in terms of apparent viscosity (mPa·s) at 290 s^{-1}, consistency coefficient K (mPa·s^n), and flow behaviour index n (dimensionless).

2.5. Ice cream analyses

Overrun (%; n = 10), firmness (N; n = 20), melting behaviour (starting time, min; rate, g/min; area retention index, A_t/A_0; shape retention index, R_t/R_0; n = 3), and colour (CIE L*a*b*; n = 6) of ice cream samples were evaluated as reported in previous papers (Alamprese et al., 2002; Moriano & Alamprese, 2017a; 2017b). In summary, for firmness a penetration test was performed by using a dynamometer (4301, Intron Ltd., High Wycombe, UK) equipped with a 100 N load cell and a stainless-steel probe (8 mm diameter) previously conditioned at −16 °C; melting behaviour was evaluated in a thermostatic chamber at 20 ± 1 °C for 90 min, by using both the gravimetric and the image analysis method; colour was measured by means of a tristimulus colorimeter Chroma Meter II (Konica Minolta, Osaka, Japan) with the standard illuminant C.

2.6. Statistical analyses

Analytical data were subjected to one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test to highlight significant differences ($p \leq 0.05$) among samples (Statgraphics Centurion 18, Statgraphics Technologies, Inc., The Plains, VA, USA). To include technological variability, results of replicated products were considered as a single sample. ANOVA and LSD were also carried out to compare the properties of the reference and optimised ice cream samples.

The experimental design data were elaborated by RSM, applying the quartic Scheffé model (Piepel et al., 2002). For the evaluation of significant coefficients, a one-way analysis of variance (ANOVA) was applied. Goodness of the models was evaluated in terms of determination coefficient (R^2), adjusted determination coefficient (adj. R^2), determination coefficient in prediction (pred. R^2), adequate precision, and lack of fit (LoF, p-value). The multi-objective optimisation of the emulsifier substitute blend was performed by applying an overall desirability function (Alamprese et al., 2007; Montgomery, 2013). DoE development and elaboration was carried out using Design Expert v. 10 software (Stat-Ease Inc., Minneapolis, MN, USA).

3. Results and discussion

3.1. Characteristics of ice cream mixes and final products

During the aging phase, important changes in the mix occur so it is important to study the effects on physical parameters to compare different formulations. The analytical results for all the mix formulations of the I-Optimal Mixture design are presented in Table 2. Density was similar (1.10–1.11 g/mL) for all the formulations, except for samples containing higher amounts of ACD (i.e., samples 1, and 8a-b), which had a significantly lower density ($p \leq 0.05$), ranging between 1.05 and 1.08 g/mL. This is possibly related to the formation of cyclodextrin aggregates by intermolecular hydrogen bonds, with a hydrophobic core (Ryzhakov, 2016; Sá Couto et al., 2018). Results for the soluble solid content are in agreement with the rationale of the formulation balance, with a grand mean of 31.7 ± 1.4 °Bx. The higher values observed in mixes with a WPP level ≥2 g/100 g (i.e., samples 3, 5, 7, and 10) are most probably related to the ash content of this ingredient, which is more than double with respect to ACD and CF (technical sheet data). The rheological behaviour evaluation of the mixes is very important for the quality characteristics of the final product and the typical shear thinning behaviour was already reported in previous works (Karaman & Kayacier, 2012; Tsevdou et al., 2019). Interesting results were obtained, highlighting a significant ($p \leq 0.05$) increase in apparent viscosity and consistency coefficient K by increasing the CF content from 0.5 to 1 g/100 g (samples 6a-b, 7, 8a-b, 9a-b, and 10). This is related to the great ability of CF in retaining water, thus affecting rheological behaviour of ice cream mixes, as already observed by Loffredi et al. (2021). Also, the pseudoplasticity of the mixes was enhanced by the increase in CF content, as demonstrated by the significant ($p \leq 0.05$) decrease of the flow behaviour index n below 0.8 in samples containing 1 g/100 g CF (samples 8a-b, 9a-b, and 10).

Table 2

Properties (mean ± s.d. values; $n \geq 3$) of the ice cream mixes produced according to the I-Optimal mixture experimental design developed for the substitution of mono- and di-glycerides of fatty acids in artisanal ice cream.

Sample	Density (g/mL)	Soluble solids (°Bx)	Apparent viscosity (mPa s)	K (mPa s^n)	n (–)
1	1.08 ± 0.02^{b}	29.9 ± 0.1^{a}	25.9 ± 0.2^{a}	49 ± 1^{a}	0.888 ± 0.002^{f}
2a	1.10 ± 0.01^{c}	30.5 ± 0.3^{a}	27.1 ± 1.6^{a}	48 ± 1^{a}	0.895 ± 0.001^{f}
2b	1.11 ± 0.01^{d}	30.5 ± 0.6^{a}	25.1 ± 0.1^{a}	45 ± 1^{a}	0.896 ± 0.010^{f}
3	1.10 ± 0.01^{bc}	32.4 ± 0.5^{cde}	29.0 ± 0.1^{b}	55 ± 1^{a}	0.887 ± 0.001^{f}
4	1.10 ± 0.01^{bc}	30.9 ± 0.8^{abc}	26.2 ± 0.3^{a}	48 ± 2^{a}	0.892 ± 0.001^{f}
5	1.10 ± 0.01^{bc}	33.8 ± 0.4^{e}	36.5 ± 1.6^{c}	94 ± 13^{b}	0.836 ± 0.017^{d}
6a	1.11 ± 0.01^{bc}	31.3 ± 0.6^{ab}	35.8 ± 0.1^{c}	78 ± 1^{b}	0.862 ± 0.001^{e}
6b	1.10 ± 0.01^{bc}	30.3 ± 0.8^{ab}	37.6 ± 0.2^{c}	89 ± 1^{b}	0.848 ± 0.001^{e}
7	1.11 ± 0.01^{bc}	34.1 ± 1.6^{e}	45.1 ± 0.2^{d}	124 ± 1^{c}	0.820 ± 0.001^{c}
8a	1.06 ± 0.01^{a}	31.6 ± 2.5^{abc}	60.4 ± 1.1^{e}	254 ± 19^{e}	0.741 ± 0.008^{a}
8b	1.05 ± 0.05^{a}	30.8 ± 0.8^{abc}	57.0 ± 0.8^{e}	219 ± 14^{e}	0.762 ± 0.009^{a}
9a	1.11 ± 0.01^{c}	32.5 ± 1.2^{bcd}	58.1 ± 0.1^{e}	208 ± 1^{d}	0.775 ± 0.001^{b}
9b	1.11 ± 0.01^{c}	31.5 ± 1.4^{bcd}	57.4 ± 0.9^{e}	202 ± 4^{d}	0.778 ± 0.001^{b}
10	1.10 ± 0.02^{bc}	33.3 ± 1.1^{de}	63.7 ± 0.3^{f}	228 ± 1^{e}	0.773 ± 0.001^{b}

K, consistency coefficient; n, flow behaviour index.

$^{a\text{-}f}$, for each variable, mean values followed by different superscript letters were significantly different ($p \leq 0.05$).

Results of the analysis performed on the ice cream samples are presented in Table 3. Lower extrusion times (ranging from 6.5 to 7.1 min) coupled with higher extrusion temperatures (above −7.3 °C) were measured for samples with a CF content of 1 g/100 g (i.e., samples 8a-b, 9a-b, and 10), according to the higher apparent viscosity and consistency coefficient values of the corresponding mixes discussed before (Table 2). The correlation between the rheological and the extrusion parameters was already reported in a previous work (Loffredi et al., 2021) and it is due to the freezer settings; indeed, the freezer extrudes ice cream when a fixed engine torque is reached, thus a higher initial viscosity of the mix implies a shorter freezing time and a higher extrusion temperature. The same samples showed lower overrun (≤41%) and significantly ($p \leq 0.05$) higher firmness (≥20 N) compared with the other formulations due, also in this case, to the very high viscosity of the mix, which made the air penetration more difficult (Goff & Hartel, 2013; Loffredi et al., 2021). Besides, these samples had the best melting performance in terms of high t_s (≥27.0 min) and significantly ($p \leq 0.05$) low rate (≤0.58 g/min), confirming results by Loffredi et al. (2021) who demonstrated the promising ability of CF as emulsifier substitute in artisanal ice cream. Indeed, the absence of CF in samples 1, 2a-b, and 3 resulted in the worst melting performance with a low t_s (≤15.5 min) and a significantly ($p \leq 0.05$) high rate (≥2.65 g/min).

Interesting results were obtained for the ice cream samples with a ACD level >2 g/100 g (i.e., samples 1 and 4), which had a relatively short extrusion time (of about 7 min) but, unexpectedly, a quite low extrusion temperature (−9.0 and −8.0 °C, respectively). This result can be probably related to a higher freezing point of the mix, which then needs lower temperatures to reach the right consistency for the ice cream extrusion.

Colour evaluation showed similar results for all the samples with a few exceptions. Samples 1 and 4, containing the higher amounts of ACD, had the significantly ($p \leq 0.05$) highest brightness (L* values) and lowest yellowness (b* values), confirming the ability of α-cyclodextrin in increasing the whiteness of samples, as declared by the producer.

3.2. Modelling the effect of the emulsifier substitutes

To better understand the single and combined contribution of the emulsifier substitutes to the mix and ice cream properties, the analytical data collected following the I-Optimal Mixture Design were elaborated by the RSM, according to the Scheffé polynomial (Table 4). Only for the melting rate a square root transformation was needed to satisfy the normal distribution of residues. Significant models ($p \leq 0.01$), mainly

Table 3
Extrusion parameters and physical properties (mean ± s.d.; $n \geq 3$) of the ice cream samples produced according to the I-Optimal mixture experimental design developed for the substitution of mono- and di-glycerides of fatty acids in artisanal ice cream.

Sample	Extrusion time	Extrusion temperature	Overrun	Firmness	Melting behaviour		Colour		
	(min)	(°C)	(%)	(N)	t_s (min)	rate (g/min)	L*	a*	b*
1	7.0 ± 0.6^{abcd}	-9.0 ± 0.1^{a}	43 ± 4^{cde}	10 ± 1^{a}	15.5 ± 1.3^{ab}	2.65 ± 0.03^{e}	93.4 ± 1.1^{e}	-3.5 ± 0.1^{cd}	5.7 ± 0.2^{a}
2a	8.3 ± 0.2^{e}	-8.0 ± 0.5^{b}	42 ± 7^{ab}	11 ± 2^{a}	12.2 ± 0.3^{a}	2.75 ± 0.22^{f}	91.9 ± 0.3^{de}	-3.7 ± 0.1^{d}	7.0 ± 0.2^{c}
2b	7.4 ± 0.1^{e}	-8.3 ± 0.3^{b}	37 ± 2^{ab}	9 ± 2^{a}	11.6 ± 1.9^{a}	3.29 ± 0.21^{f}	93.0 ± 0.3^{de}	-3.3 ± 0.1^{d}	7.5 ± 0.3^{c}
3	7.7 ± 0.4^{de}	-7.8 ± 0.3^{bc}	46 ± 8^{de}	13 ± 2^{b}	11.9 ± 0.3^{ab}	2.67 ± 0.10^{e}	90.7 ± 0.5^{abc}	-4.2 ± 0.2^{a}	7.7 ± 0.3^{d}
4	6.8 ± 1.1^{abc}	-8.0 ± 0.1^{bc}	39 ± 5^{abc}	17 ± 2^{c}	23.1 ± 2.6^{bc}	2.08 ± 0.01^{d}	93.4 ± 1.0^{e}	-2.8 ± 0.1^{e}	6.7 ± 0.2^{b}
5	7.5 ± 0.5^{cde}	-8.0 ± 0.1^{bc}	46 ± 5^{e}	9 ± 2^{a}	17.7 ± 0.8^{ab}	1.38 ± 0.07^{c}	89.9 ± 1.0^{a}	-3.8 ± 0.2^{b}	8.2 ± 0.2^{ef}
6a	7.2 ± 0.1^{bcd}	-8.0 ± 0.1^{c}	44 ± 6^{cd}	13 ± 2^{b}	22.5 ± 1.2^{bc}	0.95 ± 0.04^{b}	90.6 ± 0.9^{bc}	-3.7 ± 0.2^{d}	7.8 ± 0.1^{e}
6b	7.3 ± 0.1^{bcd}	-8.0 ± 0.1^{c}	40 ± 4^{cd}	14 ± 2^{b}	19.3 ± 0.1^{bc}	0.94 ± 0.01^{b}	92.0 ± 0.4^{bc}	-3.2 ± 0.1^{d}	8.2 ± 0.1^{e}
7	7.3 ± 0.2^{abcde}	-7.5 ± 0.5^{cd}	42 ± 3^{bcd}	10 ± 2^{a}	29.5 ± 1.0^{cde}	0.85 ± 0.01^{b}	90.5 ± 0.8^{ab}	-4.3 ± 0.1^{a}	8.3 ± 0.2^{f}
8a	7.1 ± 0.5^{bcd}	-7.0 ± 0.1^{e}	41 ± 5^{ab}	21 ± 4^{d}	33.0 ± 4.1^{e}	0.33 ± 0.03^{a}	91.2 ± 1.1^{cd}	-3.1 ± 0.2^{e}	7.1 ± 0.2^{c}
8b	7.3 ± 0.8^{bcd}	-7.0 ± 0.1^{e}	36 ± 3^{ab}	21 ± 3^{d}	46.8 ± 0.7^{e}	0.28 ± 0.06^{a}	92.2 ± 0.4^{cd}	-2.9 ± 0.1^{e}	7.1 ± 0.3^{c}
9a	6.7 ± 0.4^{a}	-7.3 ± 0.3^{de}	36 ± 4^{a}	20 ± 3^{d}	27.3 ± 4.0^{de}	0.58 ± 0.05^{a}	89.2 ± 1.2^{a}	-3.5 ± 0.2^{d}	8.3 ± 0.3^{f}
9b	6.5 ± 0.1^{a}	-7.0 ± 0.1^{de}	37 ± 3^{a}	24 ± 3^{d}	40.5 ± 0.4^{de}	0.39 ± 0.04^{a}	90.9 ± 1.1^{a}	-3.2 ± 0.1^{d}	8.2 ± 0.2^{f}
10	6.7 ± 0.3^{ab}	-6.2 ± 0.6^{f}	38 ± 5^{ab}	20 ± 5^{d}	28.7 ± 1.2^{cd}	0.49 ± 0.07^{a}	89.6 ± 0.8^{a}	-3.7 ± 0.1^{bc}	8.7 ± 0.1^{g}

t_s, starting time of melting.
$^{a\text{-}g}$, for each variable, mean values followed by different superscript letters were significantly different ($p \leq 0.05$).

Table 4
Values and significance of pseudo-coefficients (Scheffé polynomial), coefficients of determination, and lack of fit of the calculated models for the substitution of mono- and di-glycerides of fatty acids with α-cyclodextrin, whey protein concentrate with high phospholipid content, and citrus fibre.

	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}	δ_{12}	δ_{13}	β_{1123}	β_{1223}	β_{1233}	R^2	Adj. R^2	Pred. R^2	LOF (p-value)
Mixes properties																
Density (g/mL)	1.1 **	1.1 **	0.9 **	0.05 **	0.5 **	0.3 **		0.2 **	−0.6 ***				0.978 ***	0.946	0.930	0.01 n.s.
Soluble solids (°Bx)	29.3 **	32.9 **	34.4 **										0.682 **	0.624	0.518	2.63 n.s.
Apparent viscosity (mPa s)	25.9 ***	29.0 ***	161.4 ***	−5.2 n.s.	−55.6 n.s.	−42.6 n.s.				−958.1 **	384.4 n.s.	343.3 n.s.	0.996 ***	0.991	0.902	0.02 n.s.
K (mPa s^n)	36.2 ***	64.2 ***	1471.9 ***		−1314.1 *	−1408 *							0.957 ***	0.939	0.887	3.29 n.s.
n (−)	0.88 ***	0.88 ***	0.5 ***	0.1 n.s.									0.926 ***	0.904	0.878	5.48 n.s.
Ice cream properties																
Extrusion time (min)	5.4 ***	7.6 **	−0.7 **	3.7 *	17.3 **	8.0 *	−18.8 **						0.962 **	0.917	0.601	0.52 n.s.
Extrusion temperature (°C)	−6.9 ***	−7.8 ***	5.4 ***	−3.4 **	−19.3 **	−13.0 *							0.957 ***	0.927	0.810	1.00 n.s.
Overrun (%)	42.0 **	46.2 **	23.4 **										0.733 **	0.674	0.598	0.96 n.s.
Firmness (N)	11.6 ***	6.0 ***	43.3 ***										0.846 ***	0.815	0.740	2.51 n.s.
t_s (min)	16.3 ***	10.9 ***	62.4 ***										0.924 ***	0.905	0.871	2.28 n.s.
Rate (g/min)	1.7 ***	1.6 ***	3.4 ***		−7.6 **	−6.84 *							0.969 ***	0.955	0.912	2.19 n.s.
L*	93.9 ***	90.6 ***	86.8 ***										0.727 ***	0.677	0.588	0.44 n.s.
a*	−2.7 ***	−4.2 ***	−3.3 ***										0.801 ***	0.762	0.669	0.45 n.s.
b*	5.3 ***	7.8 ***	10.8 ***	3.5 **									0.917 ***	0.890	0.857	0.56 n.s.

K, consistency coefficient; n, flow behaviour index; t_s, starting time of melting.
β_0, intercept; β_1, coefficient of α-cyclodextrin; β_2, coefficient of whey protein concentrate with high phospholipid content; β_3, coefficient of citrus fibre; β_{12}, β_{13}, β_{23}, β_{123}, coefficients of the linear interaction; δ_{12}, δ_{13}, third-order coefficients; β_{1123}, β_{1223}, β_{1233}, coefficients of the quadratic terms; R^2, coefficient of determination; Adj. R^2, adjusted R^2; Pred. R^2, predicted R^2; LOF, lack of fit.
n.s., not significant ($p > 0.05$); * significant with $p \leq 0.05$; ** significant with $p \leq 0.01$; *** significant with $p \leq 0.001$.

linear and quadratic, were calculated for all the response variables and the LOF was always not significant, meaning the adequacy of the fitting. Adjusted R^2 and predicted R^2 values of each model were similar, indicating a good prediction ability of the models; the only exception was observed for extrusion time, with a difference higher than 0.20 between the two coefficients of determination; anyhow, the range of variation for this response was not large and its modelling is not that interesting due to the extrusion settings of the freezer.

The three emulsifier substitutes had a significant effect on all the quality parameters evaluated ($p \leq 0.01$), but the magnitude of their effects was different for most of the responses. As for the mix characteristics, a higher linear effect of CF was calculated for apparent viscosity and K, confirming the great ability of citrus fibre in increasing the mix thickness. However, high and negative linear interaction coefficients (β_{13} and β_{23}) were calculated for K, indicating that the presence of ACD and WPP lightened the CF thickening effect. In particular, the very high and negative value obtained also for the coefficient β_{1123} means that ACD has a great mitigating effect towards CF. Indeed, the lowest values of apparent viscosity were measured at the higher level of ACD (Fig. 1a). On the contrary, for the flow behaviour index n, the most affecting ingredients resulted ACD and WPP, which made the mixes more Newtonian with the increasing of their content.

Ice cream extrusion properties were significantly affected by the three substitutes, with a greater effect of WPP, which increased the extrusion time while decreasing the temperature; this result can be related to the higher ash content of this ingredient, already highlighted commenting the higher soluble solids of the samples containing higher level of WPP. The mineral content indeed affects the freezing point of the ice cream mix (Whelan et al., 2008), lowering it and requiring a longer freezing time to reach the correct extrusion consistency. On extrusion parameters, also the interaction between ACD and CF had a significant and synergistic effect, as well as the interaction WPPxCF for extrusion temperature. The overrun was more affected by ACD and WPP than CF, with a direct and linear effect for all the substitutes, thus confirming their ability as emulsifiers for ice creams. CF mainly contributed to the firmness and melting behaviour of the ice creams, with direct linear effects linked to the great ability of this ingredient in retaining water. Only for the melting rate also negative linear interactions of CF with ACD and WPP were calculated, indicating a smoothed effect of the combinations with respect to the presence of CF only (Fig. 1b). As for ice cream colour, a higher positive effect of ACD on L* was confirmed, whereas CF mainly contributed to increase the b* values, thus giving a more yellow colour. A significant and direct effect on b* was also observed for the interaction between ACD and WPP.

3.3. *Optimisation of the emulsifier blend*

To compare the effects of the different emulsifier substitutes with those of the commonly used MDG, and to choose the constraints for the optimisation of the emulsifier blend, a reference ice cream (REF) was produced in duplicate and analysed (Table 5). Afterwards, considering that the most important quality parameters related to the consumers' acceptability of the ice cream are the overrun, firmness, and melting behaviour (Goff & Hartel, 2013), the targets for the optimisation were defined as follows: maximising overrun; obtaining firmness, initial time of melting, and melting rate in the ranges 10–20 N, 15–18 min, and 2–3 g/min, respectively.

Applying the desirability function (Fig. 2), the best solution (desirability = 0.713) resulted in an emulsifier mixture composed of 1.1 g/100 g ACD, 1.75 g/100 g WPP, and 0.16 g/100 g CF. Using this blend, the optimised ice cream sample (OPT) was produced in duplicate and analysed, to compare the obtained results with the values predicted by the I-Optimal Mixture Design models and the REF quality characteristics (Table 5). A good agreement between the obtained results and the predicted values was observed for almost all the parameters, confirming the good predictive ability of the calculated models. The higher deviations were registered for the melting behaviour, which fell out of the target ranges for the OPT samples, but with an improvement compared to the predicted values and REF.

Comparing OPT to REF with ANOVA, the only significant ($p \leq 0.05$) differences were observed for the apparent viscosity of the mix, the melting behaviour, and the b* colour coordinate. Apparent viscosity and melting behaviour improved in OPT compared to REF. To a certain extent, a higher mix viscosity is usually related to a higher whippability and a consequent higher overrun (Goff & Hartel, 2013; Tsevdou et al., 2019). Indeed, the overrun of the OPT samples was higher than that of REF, even if the difference was not significant. The higher mix viscosity and overrun accounted also for the higher starting time of melting and the lower melting rate, providing resistance to dripping. The best melting behaviour of OPT compared to REF can be also observed in Fig. 3, in which the area and shape retention indices and some pictures of the samples taken during melting are shown. Values of b* were significantly higher in OPT than in REF; this was expected due to the positive effect of all the emulsifier substitutes already highlighted by the model study.

4. Conclusions

In conclusion, the application of DoE techniques and RSM allowed to optimise the formulation of a blend of emulsifier substitutes to obtain an artisanal ice cream of high quality and with a clean label. The right combination of ACD, WPP, and CF resulted indeed in a product with quality characteristics similar to the reference ice cream or even better. All the three considered emulsifier substitutes can be indicated in the product label without the use of the E-code, because they are classified as food fibres and proteins, with a consequent positive effect on the consumers' perception.

The application of DoE and RSM resulted fundamental to well

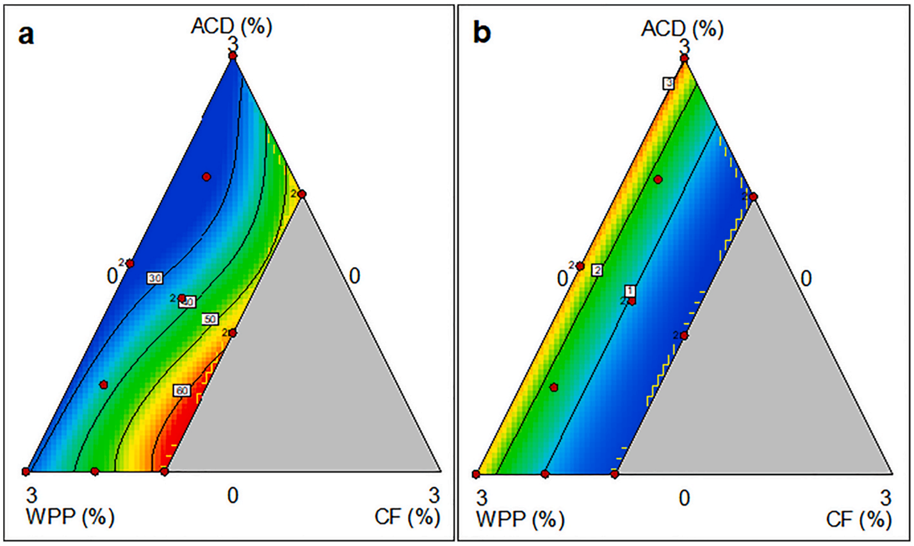

Fig. 1. Response surface for apparent viscosity (a) and melting rate (b) of ice creams prepared with α-cyclodextrin (ACD), whey protein concentrate with high phospholipid content (WPP), and citrus fibre (CF) as substitutes of mono- and di-glycerides of fatty acid.

Table 5

Mix and ice cream properties of the reference (REF) and optimised (OPT) formulations, and values predicted by the developed models (mean ± s.d. values; $n \geq 3$) for the substitution of mono- and di-glycerides of fatty acids with α-cyclodextrin, whey protein concentrate with high phospholipid content, and citrus fibre.

Sample	Density	Soluble solids	Apparent viscosity	K	n	Extrusion time	Extrusion temperature	Overrun	Firmness	Melting behaviour		Colour		
	(g/mL)	(°Bx)	(mPa s)	(mPa s^n)	(−)	(min)	(°C)	(%)	(N)	t_s (min)	rate (g/min)	L*	a*	b*
REF 1	1.07 ± 0.02	30.1 ± 0.5	24.6 ± 0.2	54 ± 1	0.864 ± 0.003	8.3 ± 0.1	−8.8 ± 0.8	36 ± 8	10 ± 2	18.3 ± 0.5	2.57 ± 0.04	92.6 ± 0.6	−3.6 ± 0.1	6.1 ± 0.1
REF 2	0.98 ± 0.05	29.2 ± 0.2	25.5 ± 0.3	61 ± 4	0.830 ± 0.030	8.2 ± 0.1	−10.2 ± 0.3	30 ± 3	9 ± 1	14.8 ± 0.3	2.70 ± 0.06	95.0 ± 0.3	−3.7 ± 0.1	6.3 ± 0.1
Mean	1.02 ± 0.06^a	29.7 ± 0.6^a	25.1 ± 0.7^a	57 ± 5^a	0.846 ± 0.020^a	8.2 ± 0.1^b	−9.4 ± 0.8^a	33 ± 4^a	10 ± 1^a	16.6 ± 2.5^a	2.54 ± 0.10^b	93.8 ± 1.8^a	−3.6 ± 0.1^a	6.2 ± 0.1^a
OPT 1	1.10 ± 0.01	31.8 ± 0.2	30.4 ± 1.1	59 ± 5	0.881 ± 0.001	7.3 ± 0.2	−8.3 ± 0.4	45 ± 4	12 ± 2	35.4 ± 1.6	1.11 ± 0.03	92.2 ± 0.5	−3.7 ± 0.1	8.3 ± 0.2
OPT 2	1.10 ± 0.01	32.8 ± 0.3	31.4 ± 0.1	60 ± 1	0.884 ± 0.001	7.4 ± 0.1	−8.8 ± 0.4	48 ± 5	14 ± 3	30.9 ± 2.4	1.14 ± 0.09	92.3 ± 0.4	−3.8 ± 0.1	8.2 ± 0.2
Mean	1.10 ± 0.01^a	32.3 ± 0.7^a	30.9 ± 0.7^b	60 ± 1^a	0.883 ± 0.001^a	7.4 ± 0.1^a	−8.6 ± 0.4^a	46 ± 2^a	13 ± 1^a	33.2 ± 3.2^b	1.13 ± 0.02^a	92.3 ± 0.1^a	−3.8 ± 0.1^a	8.3 ± 0.1^b
Predicted Values	1.10 ± 0.01	31.7 ± 0.3	30.2 ± 0.8	60 ± 7	0.883 ± 0.008	7.5 ± 0.1	−8.3 ± 0.1	43 ± 1	10 ± 1	15.6 ± 0.8	2.00 ± 0.01	91.6 ± 0.3	−3.6 ± 0.1	7.7 ± 0.1

K, consistency coefficient; n, flow behaviour index; t_s, starting time of melting.

$^{a\text{-}b}$, for the same variable, different superscript letters indicate significantly different ($p \leq 0.05$) mean values between REF and OPT samples.

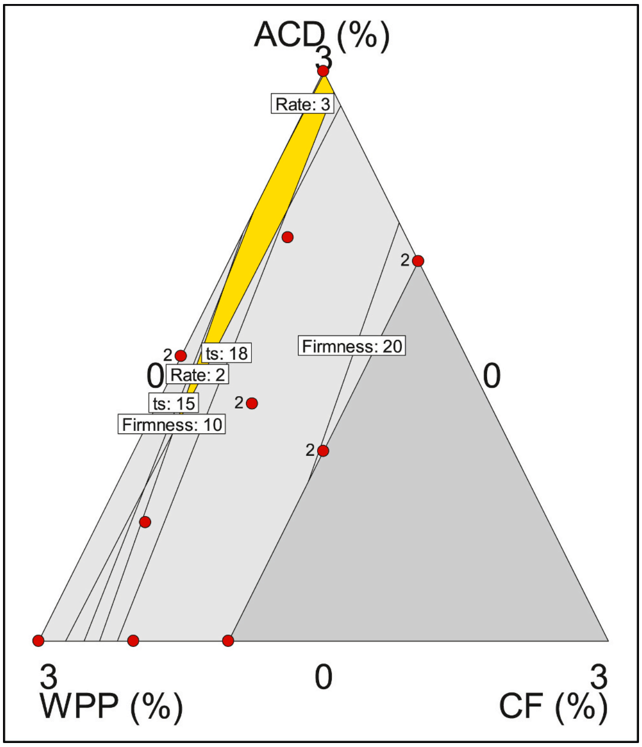

Fig. 2. Desirability plot for the optimisation of α-cyclodextrin (ACD), whey protein concentrate with high phospholipid content (WPP), and citrus fibre (CF) blend, as substitutes of mono- and di-glycerides of fatty acid in ice cream.

understand the interactions among the three tested emulsifier substitutes and to design the quality characteristics of the clean-label ice cream.

The evaluated ingredients are easily available on the market, but each marketed product can have specific properties. Thus, the results of this study should be adapted to the ingredients really used and possibly completed by sensory evaluations.

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Authors’ contribution

The Authors contributed equally to the work.

CRediT authorship contribution statement

Eleonora Loffredi: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Cristina Alamprese:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

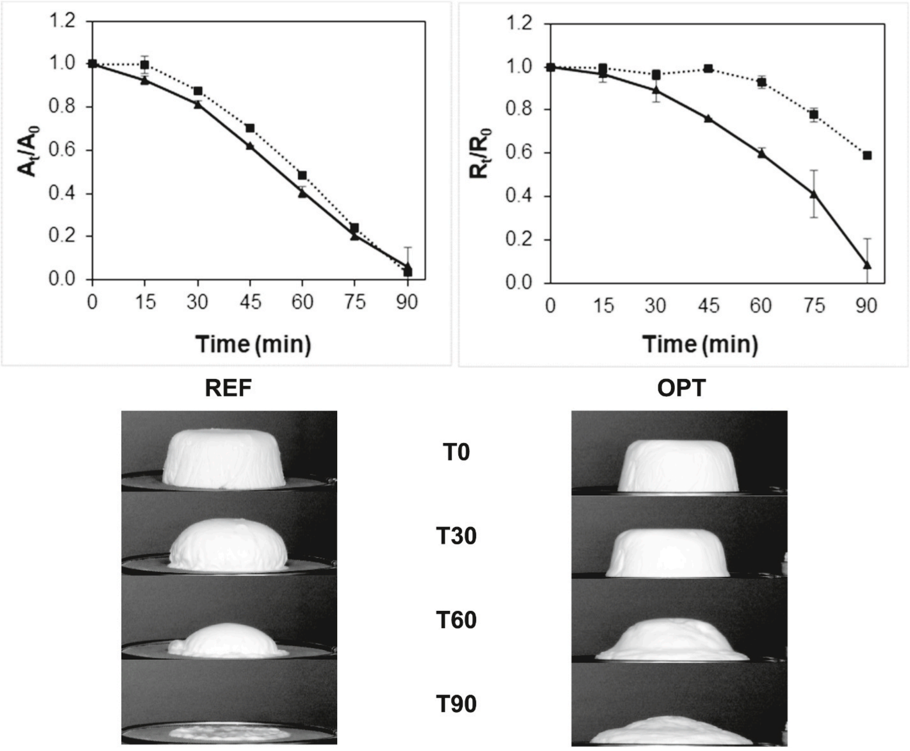

Fig. 3. Melting behaviour of the optimised (OPT; dotted lines) and reference (REF; solid lines) ice creams. A_t/A_0, area retention over melting time; R_t/R_0, shape retention over melting time. Error bars represent standard deviation values (n = 3).

Data availability

Data will be made available on request.

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