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Original article

Optimization of Low-fat Butter Using Response Surface Methodology: Effect on Physicochemical Properties and Consumers' Acceptance [1]

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Abstract

According the WHO, diets high in fat are linked to obesity and overweight, both which increase the likelihood and prospect of diabetes. Therefore the food industry has to review their formulations in relation to fat. The objective of the study was to develop and characterize a fat reduced butter. In order to manufacture product with desirable properties, formulation consisted on response surface methodology, based on 3 different factors such as percentages of emulsifier additive E471 (glyceryl monostearate, glyceryl distearate), xanthan gum (E415, thickening agent, stabiliser and emulsifier) and water, and 2 levels (-1,+1). For determination of optimum points, four responses were selected: percentages of fat, water, pH and hardness. Optimum formulas were validated by sensory tests. In the second part of this study, the effect of storage at 4°C during 20 days on physicochemical and sensory properties of the butter was assessed. Preliminary optimized formula of reduced-fat butter was obtained by emulsifier additive E471, xanthan gum E415 and water contents of 3, 0.1 and 40%, respectively. However, this fat reduction of 63% led to a weak sensory acceptance score. Additional formulation with butter aroma and coloring agent (E160a) has significantly improved consumers 'acceptance. Quality characterization showed that fat reduction in butter formula has significantly induced an increase in water activity, pH, acidity, peroxide and iodine indexes, and a decrease in hardness, when compared to control butter. Moreover, storage of low fat butter at 6°C during 20 days induced a significant decrease in pH, and iodine index, whereas acidity and peroxide indexes increased significantly and in a higher extend, when compared to control butter. Microbial load increased after 16 days of storage. These variations related to higher water content led to a decrease in low-fat butter shelf life at 6°C. Our results showed that the production of low-fat butter can be industrially applicable and recommended to people who are interested in consumption of reduced- fat foods.

Keywords: Butter, Low-fat, RSM, Quality.

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INTRODUCTION

Despite health promotion campaigns, cardiovascular diseases (CVD) and obesity are still a public health concern worldwide (Sharp et al., 2015; Couch et al., 2018). Research has clearly proved a relationship between high dietary fat consumption and rise in CVD (Wang & Hu, 2017), as well as in obesity (Bray et al., 2004). Consequently the World Health Organization (WHO) recommends individuals to limit energy intake from total fats and shift fat consumption away from saturated fats to unsaturated fats and towards the elimination of trans fatty acids. However, fats must not be banned from the diet since they are providers of calories, essential fatty acids, and fat-soluble vitamins (Mozaffarian & Ludwig, 2015). Therefore, the great demand for products with low-fat content has been and still is a challenge for the food industry and particularly, in dairy industries, since consumers demand high-quality reduced fat dairy products that are similar to their full fat counterparts. In fact, lipids contribute to product aroma, texture, appearance, flavor and mouth feel (Rønholt et al., 2013).

Recently butter is not seen unhealthy since a systematic review and meta-analysis by Pimpin et al. (2016) has indicated no significant association of butter consumption with CVD. In addition, consumers are increasingly opting for ingredients perceived as natural and less processed, including butter. Therefore in Europe, global butter consumption is rebounding after years of declines. Global retail butter sales will expand 2.9 percent to $19.4 billion in 2018, outpacing the 1.9 percent growth in sales volumes, according to Euromonitor International. This trend has also been observed in Tunisia: butter consumption has raised from 0.4 kg/person/year in 1985 to 1.0 kg/person/year in 2015 (INS, 2016). Interestingly, a survey conducted in our laboratory has shown that 76.5% of respondents expressed their motivation for the purchase of reduced fat butter (data not shown).

A fat reduction in butter can be achieved by using two methods, which can be complementary. The first method involves the removal of fat (total or partial) by including the incorporation of water into the product. This implies the addition of emulsifier agents in order to create a smooth texture, prevent separation and extend shelf life (Euston, 2008). The second method involves the replacement of fat with carbohydrate, protein or lipid-based fat replacers to ensure the functionality of the replaced fat (Roller & Jones, 1996; Lim et al., 2010). The ideal fat replacer(s) can not only reduce fat and calorie content of the product, but also maintain the quality of the full fat counterpart (Lim et al., 2010). It does not exist. However, food hydrocolloids can bring significant texturizing effect to the food products and thus can generally be expected as appropriate fat replacers. European regulation (CE/L 347/819, 2012) for fat reduced butters indicates a fat content of more than 41% but less than 61%.

Design of experiments (DOE) is an effective approach for statistical optimization (Brahmi et al., 2018). Particularly, response surface methodology (RSM) could be suggested for product formulation optimization, since it is faster, efficient and economic. In fact, the approach is based on a mathematical and statistical use of experimental data. Consequently, the technique allows to define a functional relation between one or several response of interest and a selected variables (Khuri & Mukhopadhyay, 2010). The effect of each variable as well as interaction are thus determined. On the basis of the defined model allowing response prediction, optimal conditions leading to a specific target (maximizing, minimizing or reaching specific value) could be specified (Khuri & Mukhopadhyay, 2010). To do so, desirability function could be suggested. In fact, thanks to this approach a multiple response problem could be transformed to a single response problem by means of mathematical transformations (Lazo-Velez et al., 2016).

The present study aimed to optimizing the formulation of a reduced fat butter by the use of RSM in defining the levels of water, fat replacer, and emulsifier, and that is compatible with regular butter in key sensory attributes.

Material and methods

Butter samples

Raw milk was obtained from farmers in the region of Sousse, Tunisia. Processing and sampling of butter were performed at a dairy factory in the R&D laboratory (Elbene Industries, Sousse, Tunisia). Detailed technical informations used in the manufacturing process for production of butter are intellectual property and confidential information of the Elbene Dairy Factory, as convened in a confidentiality agreement between INAT and the factory.

Storage conditions were at 6°C for 20 days.

DOE

A factorial design approach was used in this study. Responses selected were: Y1: Butter fat (%); Y2: Water content (%); Y3: Butter hardness; Y4: pH. The control factors were **X1** : emulsifier additive amount (E471), **X2** : Xantan gum amount (E415) (%) and **X3** : water amount (%) (8 experiments were conducted 2^3). Factors levels (Table 1) were selected according to preliminary tests. The following model was applied to predict the response variables:

$$y = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_{12} x_1 x_2 + a_{13} x_1 x_3 + a_{23} x_2 x_3 + a_{123} x_1 x_2 x_3 \quad \text{(Eq1)}$$

Where i variables; Y dependent variables; a_o constant term; a_i, a_{ii} coefficients; X_i linear effect; and $X_i X_i$ interactions.

Table 1. Coded levels for independent variables used in experimentation.

Variables	Units	Coded level -1	1
X1:Emulsifier additive (E471)	%	3	5
X2: Xanthan gum (E415)	%	0.1	0.3
X3:Water amount	%	40	50

Desirability function was used with a target of 63% butter fat, minimizing water content and pH and maximizing butter hardness.

Physicochemical composition analyses

Butter fat, water content, pH and acidity index were determined according to the Tunisian Standards respectively NT 14.88 (1984), 14.85 (1984) NT 14.91 (1985) and 14.83 (1983). Peroxide and Iodine indexes were carried out according to the NT 14.985 (1985) and 14.93 (1985) respectively.

Microbial analyses

Total coliforms, and yeasts and moulds were determined according to respectively Tunisian standards NT 14.130 (1998) and NT14.224 (1994).

Textural analysis

The texture of butter samples (100 g, L = 12 cm, l = 8 cm, h = 3,5 cm) was analyzed at 6°C, 1 day after their manufacturing, using a penetrometer Petrotest PNR 10 (Petrotest, 2013). Automatically within 30 seconds the needle measured the hardness sample. The texture P of the sample was read on the scale with units of mm. Triplicate were done: one in the middle, two of each side.

Sensory analysis

Quantitative descriptive analysis (QDA) of butter samples was performed by 12 trained panellists (staff from the Elbene Industrie, Sousse, Tunisia) as described in ISO 13299 (2016). Attributes were quantified with an intensity scale from 0 to 10; where 0 = attribute not detected and 10= attribute extremely strong.

Overall preference was evaluated by 36 panellists using the ranking test according to the subjects' degree of liking. A sample ballot for a ranking test of three products was adopted. A variation on this ballot lists the ranks from 1 = most preferred to 3 = least preferred (ISO 8587, 2006).

Statistical analysis

All experiments were conducted in triplicate. Values of different tests were expressed as mean ± standard deviation. SPSS 20 software was used for the statistical analysis by using a Student t-test. Significance was defined at $p < 0.05$. Minitab software (Minitab 14, USA) was used for the factorial design.

Results

Formula optimization and sensory validation of reduced fat butter

Table 2 presents the factorial design and coded levels of factors with experimental results for the studied variables. As seen, fat content ranged between 57.6 and 64%, whereas water content varied between 35.89 and 40.51%. Values for hardness were between 2.71 and 8.57 mm. For pH, at the studied range, values varied between 6.64 and 7.52. This study aimed to optimize butter formulation for reduced fat butter (63%) production with low pH and water content and maximal hardness. As shown on table 2, low levels (-1) of Emulsifier additive (E471), Xanthan gum (E415) and Water amount decreased butter pH and water content.

Table 2. Design and experimental results

Combinaison	X1: E471	X2: E415	X3: Water	Y1: Fat content (%)	Y2: Water content (%)	Y3: Hardness (mm)	Y4: pH
1	-1	-1	-1	63	35.89	3.79	6.64
2	+1	-1	-1	62.2	36.49	4.75	6.69
3	-1	+1	-1	61	37.17	2.88	6.76
4	+1	+1	-1	64	36.85	2.71	6.83
5	-1	-1	+1	57.6	40.51	8.57	6.67
6	+1	-1	+1	58.6	39.92	8.14	6.97
7	-1	+1	+1	60	38.82	6.78	6.94
8	+1	+1	+1	59	39.7	7.36	7.52

The analysis of variance (ANOVA) for fat, water content, hardness and pH has shown the significance of the model (Eq. 1).

The analytical expression correlating the studied factors (X1:Emulsifier additive (E471); X2: Xanthan gum (E415) and X3: water amount) with the different responses (Y1:Fat content, Y2: Water content; Y3: Hardness; Y4: pH) are summarized in table 3.

Table 3. Polynomial model equations and statistical parameters

Responses Yi	Equations	R^2
Y1: Fat content	$Y= 60.93+0.08X_1+0.14X_2 - 2.03X_3 -0.34X_1X_2+0.37X_2X_3+0.03X_1X_3-0.35X_1X_2X_3$	88,46%
Y2: Water content	$Y= 38.17-0.02X_1-0.10X_2 + 1.64 X_3 +0.16X_1X_2- 0.41 X_2X_3 + 0.02X_1X_3+0.36 X_1X_2X_3$	96,56%
Y3: Hardness	$Y= 5.725+0.01X_1 - 0.62X_2 + 2.10X_3 - 0.08X_1X_2+ 0.15X_2X_3 -0.11X_1X_3+ 0.24X_1X_2X_3$	98,82%
Y4: pH	$Y= 6.88+0.03X_1 + 0.11 X_2 + 0.17 X_3 -0.02X_1X_2+0.05X_2X_3+0.02X_1X_3-0.00X_1X_2X_3$	72,08%

As seen on table 3, for the R^2 was higher than 80% for Hardness, fat and water content while it was only 72% for pH.

On a second time, for each coefficient the statistical significance was evaluated with p values to obtain the reduced following models (Eq 2-5).

Y1 (Fat content) = $60.93 - 2.03X_3$ (Eq 2)

Y2 (Water content) = $38.17 + 1.64 X_3 - 0.41 X_2X_3 +0.36 X_1X_2X_3$ (Eq 3)

Y3 (Hardness) = $5.725- 0.62X_2 + 2.10X_3 + 0.15X_2X_3 + 0.24X_1X_2X_3$ (Eq 4)

Y4 (pH)= $6.88+ 0.11 X_2 + 0.17 X_3$ (Eq 5)

As shown by reduced models, fat content evolution is mainly related to water amount used. Considering butter water content, Xanthan gum (E415) negatively interacts with water amount while a positive interaction between the three studied factors. According to the reduced model, water amount increases butter hardness however Xanthan gum (E415) decreases it. Despite the contradictory main effect of both factors a positive interaction is observed between them. pH evolution is positively linked to Xanthan gum (E415) and water levels.

Formula optimization of studied variables was based on desirability function study. Optimal conditions were defined as follow: %E 415: 0.1; % E471 3%; % water 40%. These levels led to a global desirability of 86.14%.

A sensory QDA was performed in order to validate the recipe of reduced fat butter, optimized by RSM (Figure 1).

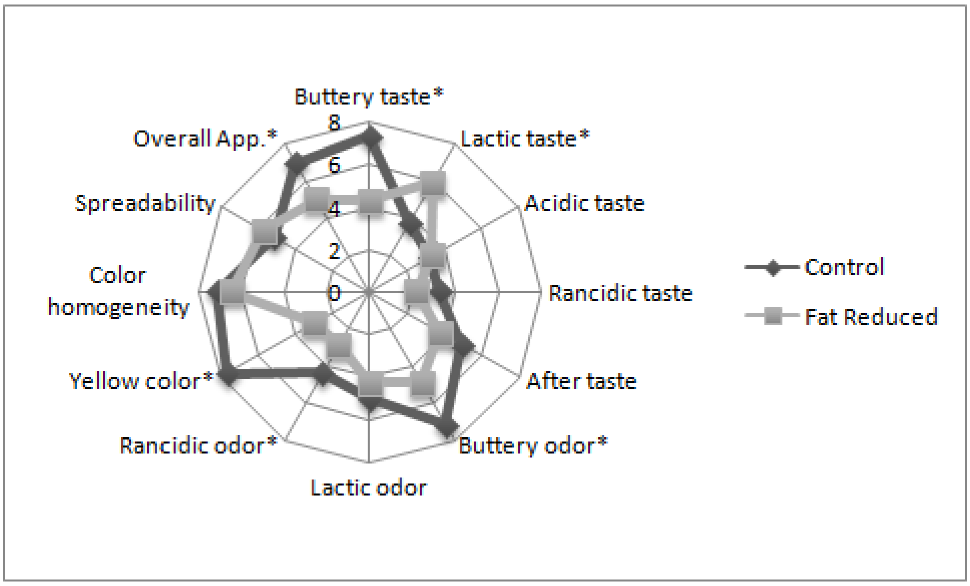

Figure 1. Effect of fat reduction on sensory quality parameters of butter (*p<0.05)

QDA indicates that fat reduction has induced a significant difference in the following descriptors: buttery taste; buttery odour; rancid odour; and yellow colour ($p<0.05$). This difference has probably influenced the overall appreciation of the products: the full fat control butter was more appreciated by panellists than the reduced fat product ($p<0.05$).

Therefore additional formulation assays have been performed in order to improve colour and flavour appreciations of reduced fat butter (data not shown). Introducing beta-carotene (E160a, 0,0035%) and butter aroma (0,13%) into optimized formula has improved consumers acceptance: 65% of the panellists have preferred the fat reduced formula than the full fat counterpart.

Effect of fat reduction and storage on quality of butter

A shown in Table 4 at day 0, fat reduction by its additional stages (mixing of the fatty phase and the aqueous phase), as well as its formulation (higher water addition), has led to a significant increase in butter pH, water content, fat, and iodine, acidity and peroxide indexes, as well as to a decrease in hardness ($p<0,05$). Coliforms were detected in reduced fat butter samples, this might be related to processing hygienic conditions.

Table 4. Quality parameters of butter affected by fat reduction and storage at 6°C for 20 days

Storage time	Product	pH	Water content (%)	P (mm)	Iodine index (g/100g)	Acidity index (mg/g)	Peroxyde index (meq O_2/kg)	Coliforms (UFC/g)	Yeasts (UFC/g)	Molds (UFC/g)
Day 0	Control	6.53 ± 0.04a,b	13.0 ±0.4a	2.14 ±0.1a	42.7 ±0.6a	0.48 ±0.06a	0.19 ±0.02a	0a	0a	0a
	Reduced Fat	6.64 ±0.03a	36.1 ±0.2b	4.04 ±0.11b	58.2 ±0.2b	1.16 ±0.08b	0.84 ±0.06b	11b	0a	0a
Day 20	Control	6.38 ±0.07b	12.6 ±0.1a	2.20 ±0.07a	38.5 ±0.4c	0.91 ±0.03c	1.08 ±0.2b	0a	0a	0a
	Reduced Fat	5.86 ±0.09c	30.8 ±0.4c	2.43 ±0.06a	22.3 ±0.5d	3.94 ±0.1d	3.49 ±0.06c	1 012c	29b	1087b

a,b,c,d letters in the same column indicate significant differences ($p<0.05$)

Two way ANOVA (product, storage time) indicated significant difference in pH, water content and hardness P as well in acidity and peroxide indexes for product factor ($p<0.05$), and in pH, water content and hardness P as well in iodine, acidity and peroxide indexes for storage factor ($p<0.05$). Moreover the interaction between both factors was significant for pH, and water content as well as for iodine, acidity and peroxide indexes ($p<0.05$).

Storage at 6°C during 20 days induced a significant decrease in iodine index, whereas acidity and peroxide indexes increased significantly and, in a higher extend in fat reduced butter, when compared to control butter (Table 4). In addition, higher microbial loads were detected in the reduced fat butter when compared to control, after 20 days of storage.

Discussion

Our study led to identify the best combination of emulsifier, fat replacer and water for a reduced fat butter, in order to get a product with a high sensory acceptance and suitable physicochemical properties. Our strategy was based on the partial removal of fat by including the incorporation of water into the product, combined to the replacement of fat with fat replacer to ensure the functionality of the replaced fat. The use of RSM approach has permitted the identification of model equations for hardness, fat and water content with a $R^2 > 80\%$ (Table 3). Consequently, these models fitted experimental and predicted values (Karazhiyan et al., 2011). Optimization was performed on the basis of desirability method. Using this optimization method allowed to incorporate desires and priorities for each of the variables. As a result of the optimization step, the best formula, obtained for the expected response value, was : emulsifier additive E471, xanthan gum E415 and water contents of 3, 0.1 and 40%, respectively. The calculated desirability for this formulation was 86.14%, and resulted reduced fat butter of good quality.

Butter texture and flavour are among quality factors influencing consumers' acceptance (O'Callaghan et al., 2016). They are largely linked to the milk fat composition and structure, and particularly to fatty acids and their degradation products (Vanbergue et al., 2018). Our results have shown that decreasing fat content in butter led to an increasing in water content, and in unsaturated fatty acids (higher iodine value), as well as to a decrease in hardness (Table 4). Unsaturated fatty acids in milk cause the butter to be softer and more spreadable (Wright et al., 2001). Beside these, because of higher water content, incorporation of xanthan gum, a fat replacer, actually denoted on the textural properties of reduced fat butter as well. Gum which was incorporated into the samples enhanced the rheological properties of the lower fat products (Williams and Phillips, 2009). In fact, Katzbauer (1998) reported that xanthan gum can act as a thickening agent, stabiliser and emulsifier. It has the ability not only to give relatively viscous solutions at low concentrations but also to stabilize the product's dispersion at rest (Roller and Jones 1996). Chronakis and Kasapis (1995) have shown that addition of xanthan to the water continuous low fat spreads stabilized the dispersion. Emulsifiers play also a role in low-fat product formulation and quality (Euston, 2008). Emulsifiers such monoglycerides were used to decrease interfacial tension between the water and the fat phase, and thus to stabilize the liquid emulsion before crystallization to secure a homogeneous product and to achieve a finely dispersed and stable water distribution, in order to create a smooth texture, prevent separation and extend shelf life (Euston, 2008). Flack (1996) reported the use of monoglycerides with high iodine value as emulsifiers, in low fat butter, for greater emulsion stability.

Interestingly, sensory QDA has not revealed a significant effect of fat reduction on butter spreadability (Figure 1). Our results are consistent with those of Brummel and Lee (1990) showing that low fat spreads containing gum had textures consistent with a high-fat cheese control. However the first formula of reduced fat butter was less preferred than a control butter, probably due to a loss of flavour. Similarly, Talbot (2016) has reported that for reduced and low fat spreads, the water phase has a great influence on the finished product in regard to taste and mouthfeel. The typical flavor of fresh butter is influenced by carbonyl compounds produced by oxidation of unsaturated fatty acids in milk fat volatile branched-chain fatty acids in butter (O'Callaghan et al., 2016). Butter colour is related to milk carotenoids which are mainly transferred into butter, contributing then to their yellow coloration. This yellow colour can be perceived as a quality attribute, according on the specific target market (Nozière et al., 2006). In addition, β-carotene has pro-vitamin A activity. Therefore, additional formulations by adding butter flavor and β-carotene, respectively, have achieved products which taste and look like butter colour, and thus have been shown to improve consumers acceptance.

Our results showed that storage has led to butter oxidation and lipolysis as shown by changes in acidity, iodine and peroxide indexes (Table 4). These changes were more drastic in reduced fat butter, probably of its high water and its unsaturated fatty acids contents (Waraho et al., 2011). As a consequence, butter texture was also affected by storage. In addition, microbial loads (ie. coliforms and molds) in reduced fat butter were after 20 days of storage, above the authorized limits according Tunisian standards NT 14.130 (1998) and NT 14.224 (1994). In fact, this observed difference was probably related to higher water content in the reduced butter, leading to an environment more favourable to microbial growth (Ledenbach,and Marshall, 2009). Processing conditions at industrial scale, contributed also in this bacterial load.

In further studies, antioxidants and preservatives need to be added to enhance the shelf life. Preservatives often used in reduced fat spreads are benzoates and sorbates (Talbot, 2016).

Conclusion

The present study aimed to developing a reduced fat butter formula in accordance with consumers' acceptance. The optimum formula of reduced fat butter was defined through the use of a factorial design approach. Moreover, the modelling of experimental data allowed the generation of useful equations for general use in predicting the quality of reduced fat butter under different combinations of factors. Optimal formula corresponded to the following amounts: emulsifier additive E471 (3%), xanthan gum E415 (0.1%), water (40%), beta-carotene (E160a, 0,0035%) and butter aroma (0,13%). This fat reduction in butter has changed its physicochemical properties, in particular its texture, as well as microbiological quality. This new formula was appreciated by 65% of the panellists. In conclusion, our study showed that the production of low-fat butter can be industrially applicable and recommended to people who are interested in consumption of reduced- fat foods.

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REFERENCES

Brahmi, M., M. Ba, Y. Hidri and A. Hassen (2018). Factorial experimental design intended for the optimization of the alumina purification conditions. J. Mol. Struct., 1157, 567-578.

Bray, G. A., S. Paeratakul and B. M. Popkin (2004). Dietary fat and obesity: a review of animal, clinical and epidemiological studies. Physiol. Behav., 83(4), 549-555.

Chronakis, L. S., Kasapis, S. (1995). A rheological study on the application of carbohydrate-protein incompatibility to the development of low fat commercial spreads. Carbohyd. Polym., 28(4), 367-373.

Couch, D., A. Fried and P. Komesaroff (2018). Public health and obesity prevention campaigns–a case study and critical discussion. Commun. Res. Pract., 4(2), 149-166.

Dietary Guidelines 2015 - 2020 (20015). https://health.gov/dietaryguidelines/2015/guidelines

Euston, S. R. (2008). Emulsifiers in dairy products and dairy substitutes. *In* Food emulsifiers and their applications (pp. 195-232). Springer, New York, NY.

Flack, E. (1996). The role of emulsifiers in low-fat food products. Handbook of fat replacers, 213-234.

INS, Institut National des Statistiques (2016). Statistiques Tunisie: Flash consommation et niveau de vie, bulletin de consommation n°1, Tunis, Tunisie, 2 p.

ISO 13299 (2016). Sensory analysis - Methodology - General guidance for establishing a sensory profile

ISO 8587 (2006). Analyse sensorielle - Méthodologie - Classement par rangs

Journal Officiel de l'Union Européenne CE/L 347/819, 2012. Matières grasses tartinables.

Karazhiyan, H., S. M. Razavi and G. O. Phillips (2011). Extraction optimization of a hydrocolloid extract from cress seed (*Lepidium sativum*) using response surface methodology. Food Hydrocoll., 25, 915–920.

Katzbauer, B. (1998). Properties and applications of xanthan gum. Polymer degradation and Stability, 59(1-3), 81-84.

Khuri, A.I. and S. Mukhopadhyay (2010). Response surface methodology. WIREs Comp stats, 2, 128-149.

Lazo-Velez, M.A., J. Aviles-Gonzalez, S. O. Serna-Saldivar and M. C. Temblador-Perez (2016). Optimization of wheat sprouting for production of selenium enriched kernels using response surface methodology and desirability function. LWT - Food Sci. Technol., 65, 1080-1086.

Ledenbach, L. H. and R.T. Marshall (2009). Microbiological spoilage of dairy products. *In* Compendium of the microbiological spoilage of foods and beverages (pp. 41-67). Springer, New York, NY.

Lim, J., Inglett, G. E., Lee, S. (2010). Response to consumer demand for reduced-fat foods; multi-functional fat replacers. Jpn J. Food Engr., 11(4), 147-152.

Mozaffarian, D. and D. S. Ludwig (2015). The 2015 US dietary guidelines: lifting the ban on total dietary fat. JAMA, 313(24), 2421-2422.

Nozière, P., B. Graulet, A. Lucas, B. Martin, P. Grolier and M. Doreau (2006). Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Tech., 131(3-4), 418-450.

NT (tunisian standard) 14. 85 (1984). Beurre-Détermination de la teneur en eau-méthode par étuvage.

NT 14. 91 (1985). Beurre-Détermination du pH.

NT 14. 130 (1998). Beurre-Dénombrement des coliformes totaux.

NT 14. 224 (1994). Beurre-Dénombrement des levures et moisissures.

NT 14. 83 (1983). Beurre-Détermination de l'acidité titrable.

NT 14. 88 (1984). Beurre-Détermination de la teneur en matière grasse.

NT 14. 93 (1985). Beurre-Détermination de l'indice d'iode.

NT 14. 98 (1985). Beurre-Détermination de l'indice de peroxyde.

O'Callaghan, T. F., H. Faulkner, S. McAuliffe, M. G. O'Sullivan, D. Hennessy, P. Dillon and R. P. Ross (2016). Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems. J. Dairy Sci., 99(12), 9441-9460.

Petrotest (2013). Manuel français : le pénétromètre PNR 10 et ses possibilités. Petrotest France, 15 p.

Phillips, G. O. and P. A. Williams (Eds.). (2009). Handbook of hydrocolloids. Elsevier.

Pimpin, L., J. H. Wu, H. Haskelberg, L. Del Gobbo and D. Mozaffarian (2016). Is butter back? A systematic review and meta-analysis of butter consumption and risk of cardiovascular disease, diabetes, and total mortality. PLoS One, 11(6), e0158118.

Roller, S. and S. A. Jones (1996). Handbook of fat replacers. CRC Press.

Rønholt, S., K. Mortensen and J. C. Knudsen (2013). The effective factors on the structure of butter and other milk fat-based products. Compr. Rev. Food Sci. F., 12(5), 468-482.

Sharp, C., P. Ball, H. Morrissey and S. Nielsen (2015). Health promotion in CVD prevention: Increasing patient awareness of modifiable cardiovascular risk factors. Australian Pharmacist, 34(3), 68.

Talbot, G. (2016). Developing food products for customers with low fat and low saturated fat requirements: dairy and meat products. *In:* Developing food products for consumers with specific dietary needs Woodhead Publishing Series in Food Science, Technology and Nutrition, Pp. 107-128.

Vanbergue, E., C. Hurtaud, J. L. Peyraud, E. Beuvier, G. Duboz and S. Buchin (2018). Effects of n-3 fatty acid sources on butter and hard cooked cheese; technological properties and sensory quality. Int. Dairy J., 82, 35-44.

Wang, D. D. and F. B. Hu (2017). Dietary fat and risk of cardiovascular disease: recent controversies and advances. Annu. Rev. Nutr., 37, 423-446.

Waraho, T., D. J. McClements and E. A.Decker (2011). Mechanisms of lipid oxidation in food dispersions. Trends Food Sci. Tech., 22(1), 3-13.

Wright, A. J., M. G. Scanlon, R. W. Hartel and A. G. Marangoni (2001). Rheological properties of milkfat and butter. J. Food Sci., 66(8), 1056-1071.