

DEVELOPMENT OF A COOKIE ADDED WITH OMEGA 3 FATTY ACIDS SOURCE AS FUNCTIONAL FOOD

DESARROLLO DE UNA GALLETA DULCE ADICIONADA CON FUENTE DE OMEGA 3 COMO ALIMENTO FUNCIONAL

ABSTRACT

The aim of this work is to develop a cookie added with omega 3. Three commercial forms of omega 3 is evaluated as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (emulsion, powder and oil) and different omega 3 (ω 3): omega 6 (ω 6) ratios (1:5, 1:8, 1:10). In general for the study is used a multistage factorial system, which evaluates the rheology of the dough and the texture, moisture, color, a_w of the cookies after baking. During the storage the concentration of ω 3 is evaluated as linolenic acid, ω 6 as linoleic acid, DHA, EPA and the sensory attributes of the final product. During the elaboration of the dough the commercial forms of ω 3 with better performance in the rheological test are the emulsion and oil forms, with 1:8 and 1:10 ratios, while in the elaboration of the cookies the better combination is emulsion form and 1:10 ratio; which is stored under extreme conditions of storage ($40 \pm 2^\circ\text{C}$ y $75 \pm 5\% \text{ RH}$) for a 60 days period, and presented good acceptance from a semi-trained panel and a decreasing concentration of ω 3 fatty acids as DHA + EPA of approximately 6%

Key Words: Functional Food, omega 3, DHA, EPA, cookies

RESUMEN

El objetivo de este trabajo es desarrollar una galleta dulce adicionada omega 3. Tres formas comerciales de omega 3 se evaluaron como ácido docosahexaenoico (DHA) y ácido eicosapentaenoico EPA (emulsión, polvo y aceite) y diferentes relaciones omega 3 (ω 3): omega 6 (ω 6) (1:5, 1:8, 1:10). En general se utiliza un sistema factorial polietápico, en el cual se evalúa la reología de las masas, y la humedad, textura, color y a_w de las galletas después del

horneado. Durante el almacenamiento se evalúa el contenido de $\omega 3$ como ácido linolenico, $\omega 6$ como ácido linoleico, DHA, EPA y los atributos sensoriales del producto final. Durante la elaboración de las masas, las formas de $\omega 3$ que mejor desempeño presentan en las pruebas reológicas, son las formas de emulsión y aceite con las relaciones 1:08 y 1:10, mientras que en la elaboración de las galletas, la mejor combinación es emulsión y relación 1:10; la cual se almacena en condiciones extremas ($40\pm 2^{\circ}\text{C}$ y $75\pm 5\%$ HR) por un periodo de 60 días y presenta buena aceptación por parte de un panel sensorial semi-entrenado y una disminución de la concentración de los ácidos grasos ω -3 como DHA + EPA, de aproximadamente 6%.

Palabras claves: Alimentos funcionales, omega 3, DHA, EPA, galletas

INTRODUCTION

The conception of healthy food over the world has been evolving throughout the time. People know which are the health risks they are facing and the actions how they can be prevented, due that governments have been developing campaigns in order to inform the community the risk of suffering diseases. One of the more common illnesses in our time is from cardiovascular nature, understood as problems related with the heart and that can be expressed in different ways as hypertension, arrhythmia, thrombosis and arteriosclerosis.

In developed countries is believed that consume of lipids can contribute between 30 – 40% of the total energy of the human been, where the unique specific request of fat in the diet, are the essential fatty acids as ω -3 and ω -6 (1).

The $\omega 3$ fatty acids are physiologically active compounds (PAC) that have an important relevance in the cholesterol reduction (2), promote the formation of compounds with a low inflammatory activity and powerful platelet aggregation inhibitors, which have an effect opposite to those responsible for common cardiovascular diseases (3).

Recent studies suggest that an $\omega 3$ above of 8% is a reasonable value that can reduce the risk of suffering cardiovascular diseases (4), besides the American heart association has approved the use of omega 3 with a 1g/day dose with a combinations of DHA and EPA, for patients with cardiovascular problems (5), the Food and Drug Administration (FDA) sets that the consume of DHA and EPA should not exceed 3 g/day in the fish oil way, food and dietary supplements. However the typical recommendations are from 0.3 to 0.5 g/day (6). The 288 resolution of 2008 from the Ministry of Social Protection in Colombia does not setup a diary reference value for this component, and sets general conditions with the declaration of properties related with the nutrients content, in the article 16, numeral 16.2, where should be declared “Contain (quantity) g of omega 3 fatty acids per portion”.

The clearest association of these fatty acids with the prevention of suffering cardiovascular diseases is, that they are precursors of prostaglandins (I_3), leukotrienes (B_5) and thromboxanes (A_3) of the series 3 and 5, cyclooxygenase and oxygenase way, that are involved in the inflammation regulatory, platelet aggregation and vasoconstriction/ dilatation, with much less intensity responses that other precursors as omega 6 (Araquidonic Acid) (7).

All these studies make the societies every day, search for new alimentation systems that prevent or reduce the risk of suffering from these degenerative diseases which are against health, well-being and that can even cause death to people.

In the baking sector, the application of functional food has not been well developed. The sectors more purchased in this category are confectionary and dairy; it does not imply the baking industry not provide a good matrix for this kind of products, with good flavor and healthy benefits (8).

However studies developed by some authors shows that the functionality of raw materials within the study matrix is of too much relevance, because this can impact directly the quality

of the final product. Specific for this topic there are studies where is evaluated the behavior of refined oils as girasol in cookies, is evaluated fat replacement with structured lipids, fiber, proteins and carbohydrates and others, where shows changes in the final product (9 - 12), because of this, it is necessary to observe the incidence that can have this PCA in the product.

The aim of this study was to develop a cookie added with $\omega 3$ fatty acids (DHA – EPA) under a preparation methodology and process as technological model that allows obtaining a product with an excellent flavor and health benefits.

METHODS AND MATERIALS

Raw materials

It was used for the elaboration of cookies: soft wheat flour, red winter variety from United States with a 11.6% of moisture and prox. 8% of protein, vegetable shortening palm (VSP) (Grasco®) 99% solids, rolled oats (Pronalce®), water, raisins (Alba®), standard sugar (Manuelita®), salt (Refisal®), pasteurized whole egg (Avinal®) and chemical leavenings (Bellchem®), sodium acid pyrophosphate (98% purity), sodium bicarbonate (purity > 99%) and ammonium bicarbonate (purity > 99%).

The omega 3 types that were used are the following: ROPUFA® 10 n-3 (ω -3P) powder from DSM, contains approximately 30% of omega 3; eicosapentaenoic acid (C20:5 $\omega 3$, EPA) and docosahexaenoic acid (C22:6 $\omega 3$, DHA), modified starch, sodium ascorbate and silicon dioxide. OMEVITAL 1812 TG GOLD (ω -3A) oil from Cognis Nutrition and Health with approximately 26% of DHA + EPA, and DENOMEGA GAT 100 (ω -3E) emulsion from GAT Food Essentials with approximately 12% from DHA+ EPA, that it has a combination of $\omega 3$, technological additives (emulsifier, antioxidant) and water.

Physicochemical Characterization

It were performed tests of moisture under the norm AOAC 925.45 (13), water activity (a_w) with a dew point hygrometer to 25°C (Aqualab Decagón TE serie), refractive index under the AOAC 921.08 (13), iodine value (IV) through the AOAC 920.158 (13), and peroxide value (PV) under the AOAC 965.33 (13). The quantification of linolenic acid, linoleic acid, DHA and EPA were performed under the AOCS Ce 1b-89 method (14) and by un accredited laboratory (TECNIMICRO, Medellín), with a gas chromatograph Shimadzu 2014 model, capillary column SHX70 Shimadzu, 0.25 mm inner diameter and 0.25 μ m inner layer; the fat extraction was performed in cold conditions to avoid the damage of the fatty acids.

Physical Characterization

Color was determined using the spectrophotometer X-RITE SP64 model with a D65 illuminant and 10° observer as reference. From this reflection spectrums it was obtained the coordinates of color CIE-L*a*b*.

The texture was determined form fracturability test with the texture analyzer TA.XT2 Stable Micro Systems (SMS), using a load cell of 25 kg, the tests were set with a penetration speed of 2mm/s and 30mm distance. The fracture force was defined at the point where it produces an appreciable drop of the force cause for the partial or total rupture of the sample.

The alveographic measures were performed using the (AACC 54-30) method (15) and the Alveolink-NG software developed by Chopin S.A. (Villeneuve La Garenne, France). The results of the alveograms include the maximum over pressure tenacity (P) measure in “mm” which are necessary to blow the bubble dough (resistance to the extension index); the average of the abscise extensibility (L) measure in “mm” for the rupture of the bubble (dough extensibility index); the deformation energy (W) measure in “J” and the relation of the configuration curve (P/L) (gluten performance).

The alveographic measures were performed with a 250 g of flour and 121 mL of the saline solution with a 2.5% concentration, calculated from the initial moisture of the flour, and the

omega 3. The last was mixed before the tests and at the end all the doughs contained the same percentage of ω -3.

Sensory Evaluation

The sensory analysis was performed with a semi-trained panel of judges from the INTAL foundation (Medellín), through the quantitative response test under the NTC-5328 norm (16) at 0, 30 and 60 days of the stability study in accelerated conditions. The characteristics: odor, objectionable odor, hardness, crunch, oat flavor, sweet flavor and objectionable flavor were evaluated with an intensity scale of 7 points, where (0) = absent, 1 y 2 (low), 3 (medium low), 4 (medium), 5 (medium high), 6 and 7 (intense).

Storage

The accelerated storage of the cookie was performed with an environmental chamber Dies C480E 20512 series, where the final product was subject to extreme conditions of temperature ($40 \pm 2^{\circ}\text{C}$) and relative moisture ($75 \pm 5\%$). Besides it was evaluated the concentration of ω -3: ω -6, DHA and EPA at the time 0, 30 and 60 days of the storage. The packing material that was used for the stability test was a metallic BOPP (polypropylene biooriented) from Alico S.A.

Process

The technology used for the elaboration of the cookies was wire cut and agglutinant dough. The doughs were prepared with 1:8 and 1:10 ratios of ω -3: ω -6 and different forms of ω -3 (oil and emulsion), after performance behavior of the doughs in the alveographic tests. The mix of the ingredients was performed in three steps: mix of vegetable shortening palm with ω -3, egg, water, sugar and leavenings (high speed), after the flour is added; the last ingredient is the oat with the raisins (low speed). The cookies were subject to two baking processes; 150°C -8 minutes and 160°C -7 minutes, using an industrial convection oven with a gas as heat source, Talsa brand. The selected cookie was taken under extreme storage conditions during 60 days to observe the stability of the PAC and the moisture absorption.

Experimental Design

The experiment was performed following a diagram of multistage factorial design, which has the following phases:

1. Interaction of raw materials with ω -3

1.1. PV: In this phase the factors were the type of ω -3 with levels (emulsion, oil and powder) and ω -3: ω -6 ratios with levels (1:5, 1:8, 1:10). It was performed 4 replicas and 3 repetitions each one.

1.2. Dough Alveograms: The factors considered for this stage were the type of ω -3 with levels (emulsion, oil and powder) and ω -3: ω -6 ratios with levels (1:5, 1:8, 1:10). It was performed 4 replicas and 3 repetitions each one. The table 1 resumes the experimental design of Dough Formulations for alveographic measurement.

Table 1. Dough Formulations for Alveograms

Ingredients	Formulations																	
	ω 3E 1:05		ω 3E 1:08		ω 3E 1:10		ω 3A 1:05		ω 3A 1:08		ω 3A 1:10		ω 3P 1:05		ω 3P 1:08		ω 3P 1:10	
	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%
Flour (11,6% moisture)	250	63.7	250	64.6	250	65.8	250	64.7	250	65.4	250	66	250	65	250	65.6	250	66.1
Omega 3	21.6	5.5	15.9	4.1	9.05	2.4	15.3	4	11.3	3	7.72	2	14	3.6	10.3	2.7	7.03	1.9
Saline Solution (2,5%)	121	30.8	121	31.3	121	31.8	121	31.3	121	31.6	121	32	121	31.4	121	31.7	121	32

2. Formulations of cookies with ω 3. For this stage it was taken as factors: type of ω -3 with levels (emulsion and oil) and ω -3: ω -6 ratios with levels (1:8 and 1:10). The response variables were: moisture, fracturability, a_w y color. It was performed 3 replicas and 7 repetitions each one.

3. Stability design and sensory analysis: In this stage it was taken just one combination of a cookie (emulsion, 1:10 ratio) which was performed 3 repetitions for each response variable (quantity of ω -3, moisture and sensory analysis).

For the data analysis was used the statistical software R.

RESULTS AND DISCUSION

Raw Material Characterization

The table 2 and 3 present the average values and the standard deviation of the physicochemical and mechanic characteristic of the principal raw materials.

Table 2. Wheat Flour Characterization

Raw Material	Tenacity (mm)	Extensibility (mm)	10^{-4} Force (J)	Balance
Wheat Flour	34.8 \pm 3.9	109.5 \pm 10.1	108.1 \pm 14.1	0.32 \pm 0.06

Table 3. VSP, Oat, Egg, and Polyunsaturated Fatty Acid Characterization

Raw Material	PV (meq O ₂ / g)	IV (g Iodine/100 g)	Reflective Index	Linolenic acid (%)	% Fatty Acids DHA + EPA	Linoleic acid (%)
VSP	0.32 \pm 0.13	61.96 \pm 0.40	1.46 \pm 0.01	0.30 \pm 0.01	---	10.58 \pm 0.30
Oat	---	---	---	0.025 \pm 0.010	---	0.99 \pm 0.30
ω -3P*	---	---	---	30.00 \pm 1.40	25.00 \pm 0.90	---
ω -3A*	---	---	---	41.30 \pm 2.40	27.5 \pm 0.14	---
ω -3E*	---	---	---	11.35 \pm 0.50	9.42 \pm 0.45	---
Egg	---	---	---	0.05 \pm 0.01	---	1.90 \pm 0.20

The results of the characterization of the flour, in terms of dough rheology are according to Wang *et al.*, 2002 (11) with tenacity values of 48mm, extensibility 107mm, force 119 x10⁴ J and balance 0.5.

The table 4 presents the average values of the PV and standard deviation of the mix between VSP and PCA.

Table 4. PV for the mixes of VSP + ω -3

Ratio	VSP + ω -3P			VSP + ω -3A			VSP + ω -3E		
	1:05	1:08	1:10	1:05	1:08	1:10	1:05	1:08	1:10
PV (meq O ₂ /g)	0.37±0.04	0.32±0.02	0.36±0.02	0.42±0.01	0.53±0.03	0.45±0.02	0.54±0.03	0.52±0.01	0.49±0.01

The PV of the different types of ω -3, according to the ratios chosen for the study presented significant difference ($p < 0.05$) for the ANOVA by the effect type of ω -3. The emulsion type presented the highest values (≈ 0.5 meq O₂/Kg), follows for the oil type (≈ 0.4 meq O₂/Kg) and the last was the powder kind (≈ 0.3 meq O₂/Kg).

PV measures the degree of oxidation which has been subject to fat or oil (12). The values of PV and IV of the VSP used, have the normal average values for this kind of multipurpose fats, PV (0.2 – 0.6 meq O₂/ g), IV (65 – 80 g Iodine/100 g) (12). Verardo *et.al.*, 2008 (17) in the study of lipid oxidation in spaghetti with ω -3 fatty acids described how the propagation period in lipid oxidation proceed considerably faster for this product through the storage time, nevertheless at the end of the study did not present significant differences compared with the control. This information allows to suggest that even the ω -3 degrade faster than others fatty acids, at the end of the storage the peroxide value will be the same for products without this PAC.

However it is important to note that as the lipid is oxidized its concentrations falls and the amount of hydroperoxide increases to a maximum, but it falls subsequently. So the measure of oxidation based purely on peroxide value can be confusing. The volatile products, which are derived lipids, then rise slowly (18).

Dough with $\omega 3$ Characterization

The Figure 2 presents the average values and the standard deviations of the parameters of alveograms by function of ω -3 and ω -3: ω -6 ratio. The ANOVA presented significant difference ($p < 0.05$) in all the variables by the effect of ω -3 type and ω -3: ω -6 ratio.

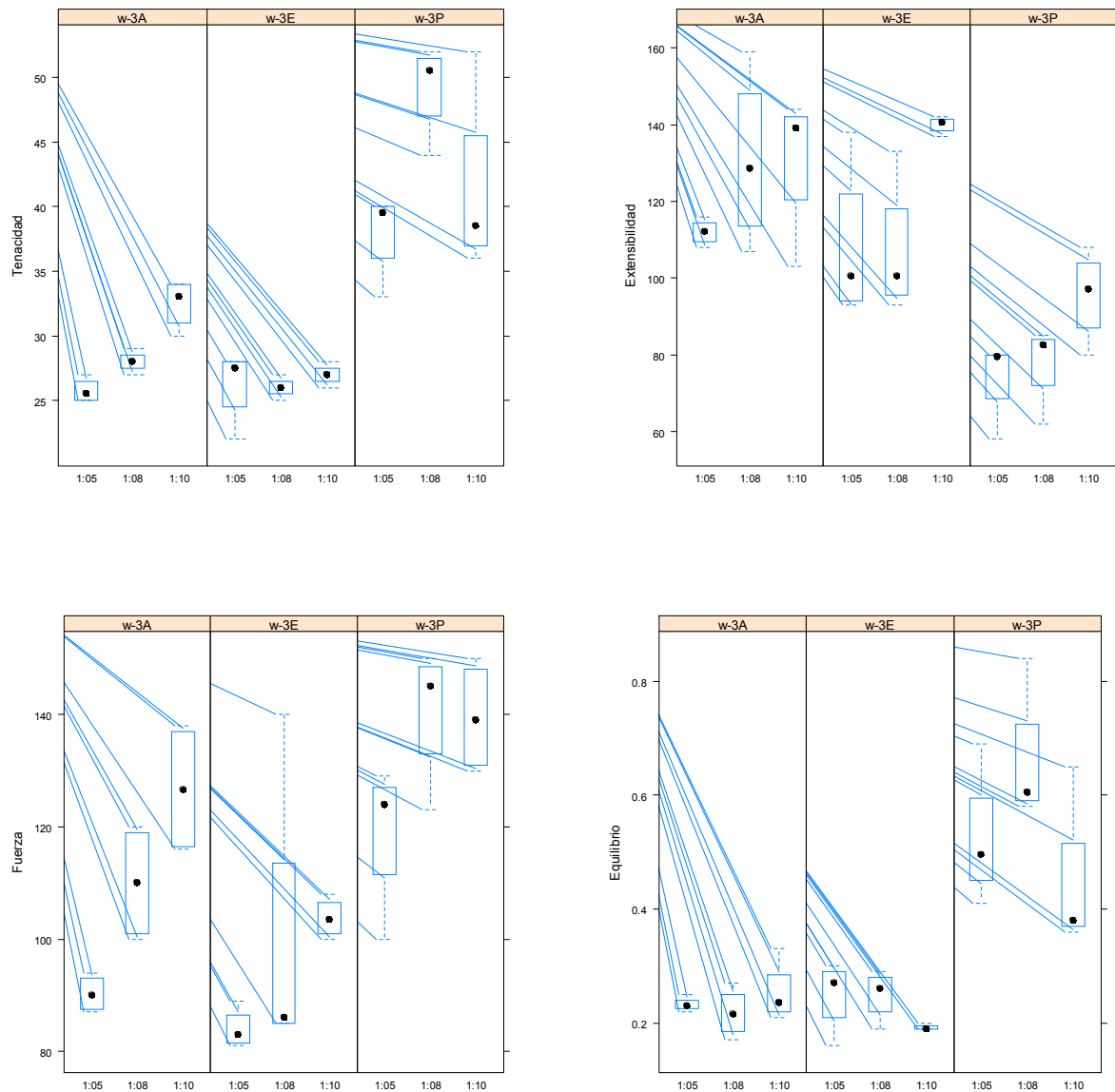


Figure 2. Dough Alveograms. ω -3E= Emulsion, ω -3A= Oil, ω -3P= Powder. Relations ω -3: ω - 6, 1: 05, 1:08, 1:10

For all the cases, the type ω -3P gives more tenacity (P) to the dough, compared with the ω -3A y ω -3E and reaches a maximum point at the 1:8 ratio.

For the type ω -3A the tenacity increases whit the decrease of the ω -3: ω -6 ratios, while the type ω -3E, have a tendency to remain constant; however the values do not get away from the tenacity average presented by the flour that do not contain this kind of polyunsaturated fatty acids (See Table 2).

The lipid / protein relation propose by Pomeranz and Chung, 1978 (19), suggest that the doughs are strengthened by the capacity of polar lipids to associate with the protein and the starch (20); This phenomenon can be observed with the ω -3 powder, because of its composition with starch (polymers), which is interacting in the protein-lipid-starch complex, making it stronger than the other two types of ω -3, with higher tenacity values.

Researches preformed by Addo *et al.*, 1995 (21) with sucrose esters fatty acids in hard red winter wheat flour have shown a decrease of the tenacity with the increase of these fatty acids.

For all the cases, the ω -3A gives more extensibility (L) to the doughs, follow by the types ω -3E and ω -3P, where the last decrease the values; which can indicate that the fluid type used, impact directly in a more elastic dough. Song and Zheng., 2007 (22), cite to Watanabe *et al.*, 2003 (23) and explain that this could be due to the fat, that when it is in a solid condition extends until the limit of tension, where the structure of the network of gluten start to brake and particularly the addition of non polar lipids facilities the aggregation of gluten and gives a more elastic behavior to the doughs, which can be confirmed with the higher values of extensibility presents by the ω -3A (See Figure 2). The decrease of the ω -3: ω -6 ratios (< addition of ω -3 to the mix), increase the elasticity of the doughs.

For the force (W) case while the relation ω -3: ω -6 decrease, the values increase. The values with the type ω -3A are closer to the characterization and the type ω -3E slightly decreases the parameter. However the change in force is the result of its dependency form P and L, so the change is not going to be a true response by the factors, as the two other parameters are (21).

The balance of the doughs, which is the product of the relation between tenacity and extensibility (P/L); the ω -3P type gives higher values, due the high tenacities and low extensibilities. Studies have found that there is a positive correlation between (P/L) and the diameter of the cookie (10, 24). This could predict that with the ω -3P would be obtained a smaller diameter from the normal.

All these analysis suggest that the types of ω -3 can continue to the next step of elaboration and baking the cookies, are the ω -3A y ω -3E types and the 1:8 y 1:10 ratios, because these combinations present closer values to the averages found in the flour characterization and help to the functionality of the doughs.

Cookies

Moisture

The Figure 3 presents the average values and the standard deviation of the moisture of cookies elaborated with ω -3, function of ω -3 type, ω -3: ω -6 ratios and baking processes. The ANOVA presented significant difference ($p < 0.05$) in this variable by the effect of ω -3 type and process, besides there is an interaction between relation and type.

For all cases, the ω -3A type gives higher moisture to the cookies compared with the ω -3E type. Parallel when the baking process at 150°C- 8 minutes dehydrated less the product compared with the process at 160°C- 7 minutes where the moisture decrease.

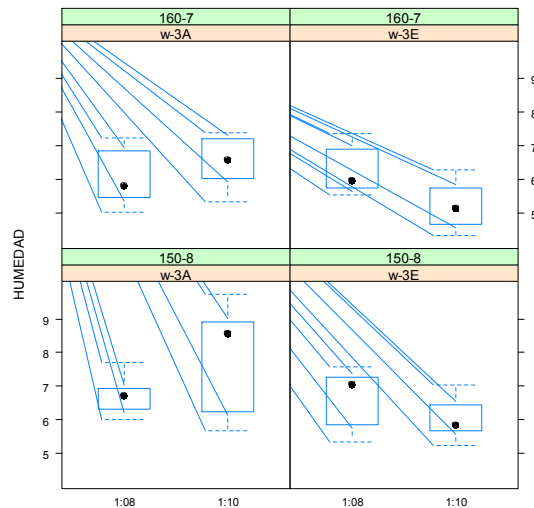


Figure 3. Moisture Results. ω -3E= Emulsion, ω -3A= Oil. Relations ω -3: ω -6, 1:8, 1:10. Process types 150°C – 8minutes, 160°C – 7minutes.

On the other hand when ω -3: ω -6 ratios decrease, impact directly increasing the moisture with the ω -3A and decreasing with ω -3E.

This behavior could be due to the importance that it has the type of fatty acids added to the wheat flour, because this can affect the formation of the lipid-amylose complex which influences the dough viscosity (25).

The effect to increase the lipids content produce a decreasing of the dough viscosity which can be reflected in higher moistures (26,27), similar to the behavior shown by the ω -3E type, besides studies suggest that when fat is disperse into a food material, hampers the migration of water in a solid matrix, decreasing the effective diffusivity of moisture (28), this could explain why with the ω -3E when there is more quantity of lipids, the moisture increase as

well; the contrary happen with the ω -3A which does not have this properties and induce the contrary effect.

Emulsifiers on the other hand in bakery are compounds that specifically facilitate the formation or stabilization of one liquid phase into another liquid phase, acting as surfactants that mainly interact with gluten and crumb softeners or anti-firming agents that can complex gelatinized starch (29). This is the reason why the cookies are softer whit the ω -3E.

This suggest that the behavior of the ω -3A, besides that is different to the expected and what is found in the literature, has a unstable performance at the 150°C - 8 minutes process in the 1:10 relation due to the higher deviation, compared with the 160°C - 7 minutes process.

a_w

The figure 4 presents the average values and the standard deviation of a_w of the cookies with ω -3, in function of the ω -3 type, ω -3: ω -6 ratios and baking process. The ANOVA presented significant difference ($p<0.05$) by the effect of the ω -3 type and there is an interaction between process and relation.

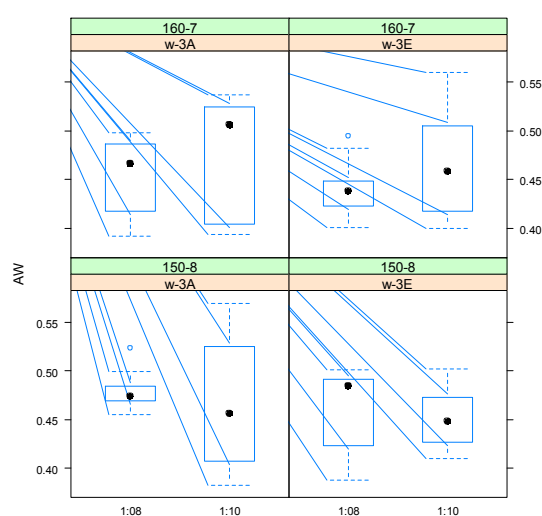


Figure 4. Results of a_w . ω -3E= Emulsion, ω -3A= Oil. Relations ω -3: ω -6, 1:8, 1:10

Process type. 150°C - 8 minutes, 160°C – 7 minutes.

The ω -3A increases the response variable and the ω -3E decrease. For the low relations, the process at 160°C – 7 minutes increase the values of a_w and the process at 150°C – 8 minutes decrease it. This behavior suggests that the time influences more in the free water extraction that the temperature of baking.

Texture

The Figure 5 presents the average values and the standard deviation of the cookie with ω -3 fracturability, in function of the ω -3 type, relations of ω -3: ω -6 and process. The ANOVA presents significant difference ($p < 0.05$) by the effect of relation and process.

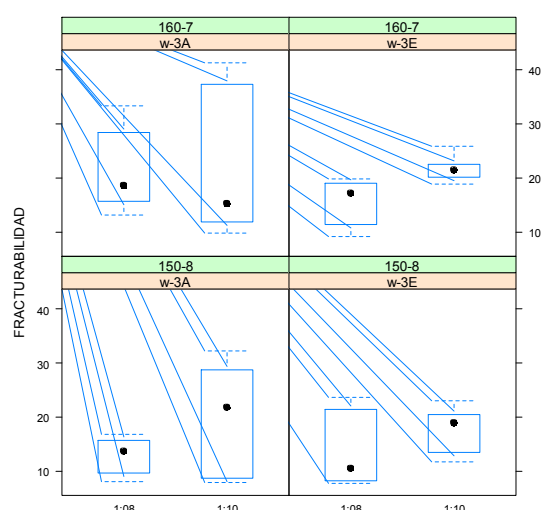


Figure 5. Results of cookie fracturability. ω -3E= Emulsion, ω -3A= Oil. Relations ω -3: ω -6, 1:8, 1:10. Process Type. 150°C - 8 minutes, 160°C – 7 minutes.

For all the cases when ω -3: ω -6 ratios decrease, the fracturability of the cookies increase. For the process at 150°C - 8 minutes the fracturability is lower and for the process at 160°C- 7 minutes is higher.

Greethad, 1969 (30) cited by Jacob and Leelavathi, 2007 (9) suggests that more plastic and soft fat is, better its performance. A softer fat, gives less fracturability to the cookie; in general the plasticity of the fats is necessary in the cremated stage to trap air, resulting an important leaving effects and softer cookie. On the other hand the oils are dispersed in a globules form trough the dough and they are less effective in their softening and aerating properties (9). This explains why the cookies with ω -3A are harder (superior fracturability) than the cookies elaborated with ω -3E, which contain emulsifier in their composition and have the capacity to trap air and improve the cremated system properties.

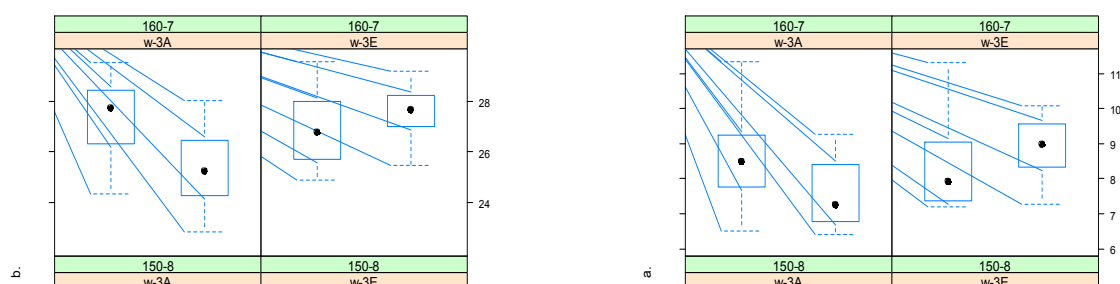
The highest level of this component, the products that have them make them softer. On the contrary if they have a low level, the products get harder (31).

The high dispersion of the data with the ω -3A and 1:10 ratio (See Figure 3), can indicate that low dosages do not dispersed well through the whole matrix and this reflect the variability of the fracturability in cookies.

Color

The Figure 6 presents the averages values and the standard deviation of the color in cookies with ω -3 in function of the ω -3 type, ω -3: ω -6 ratios and baking process.

The ANOVA presented significant difference ($p < 0.05$) by the effect of the ω -3 type and there is an interaction between process and ratio. However the variations are small, and barely perceptible to the human eye. The dispersion of the data is due to the rugosity of the cookie which influence directly in the measurement.



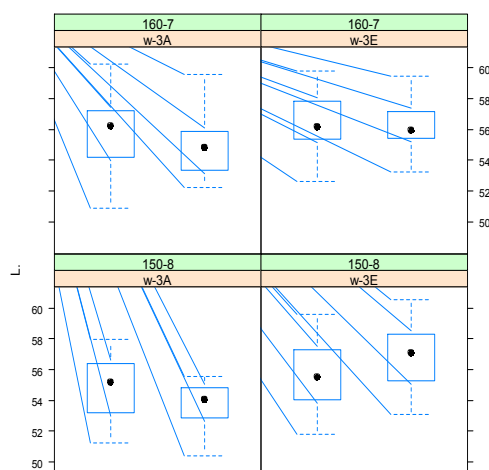


Figure 6. Results of color. ω -3E=Emulsion, ω -3A=Oil. Relations ω -3: ω -6, 1:8, 1:10. Process Type. 150°C - 8 minutes, 160°C – 7 minutes.

According to the results in this stage, is determined the ω -3E at 1:10 ratio and the process at 160°C - 7 minutes, presents a better behavior and results stability.

Stability

The Table 5 presents the average values and the standard deviation of the ω -3 fatty acids concentration during 60 days of storage in extreme conditions of temperature ($40 \pm 2^\circ\text{C}$) and relative moisture ($75 \pm 5\%$).

Table 5. Fatty acid concentrations in different storage times

Time (Days)	Linonelic acid (%)	DHA (%)	EPA (%)	Linoleic acid (%)
0	0.290 ± 0.005	0.066 ± 0.005	0.106 ± 0.005	2.246 ± 0.010
30	0.250 ± 0.005	0.060 ± 0.001	0.100 ± 0.001	2.170 ± 0.010
60	0.250 ± 0.005	0.063 ± 0.005	0.103 ± 0.005	2.160 ± 0.010

The ANOVA presented significant difference ($p < 0.05$) by the effect of time over the response variables ω -3 and ω -6, decreasing the values when the storage time increase. There is not a significant change in the DHA + EPA content.

It could be determine that the DHA and EPA lost are approximately 6% from the initial theoretical dose, similar to a study performed by Sadiq *et al.*, 2007 (32), where the lost of vitamin A before and after baking of a fortified cookie was about 8.69 – 11.1%.

The cookie approximately contribute with the 4.80% of ω -3 and 2.72% of DHA + EPA per 26 g portion of cookie (product presentation) from the suggest recommendation by the FDA (1.6 g/day). Another organism as the OTAN suggest that the requirements can be between 0.3 – 0.5 g/day of ω -3 (6); for this case the portion contribute with approximately 19.5% ω -3 and the 11.05% of DHA + EPA.

The Table 6 presents the average values and the standard deviation of the cookies moisture during the storage time. The ANOVA presents significant difference ($p < 0.05$) by the effect of time.

Table 6. Cookie moisture during storage

Day	6	8	15	22	29	36	43	50	57
Moisture (%)	4.57±0.04	4.64±0.01	4.77±0.04	4.83±0.01	4.91±0.01	5.10±0.02	5.22±0.02	5.34±0.01	5.40±0.04

The values increase when the storage time increase, which is an expected behavior; the values are according with those obtained in the process and they do not negatively influence the sensory evaluation of the products, as can be seen with later results (Table 7).

Table 7. Sensory attributes of ω -3 added cookies.

Time (Days)	Color	Characteristic odor	Objectionable odor	Hardness	Crunch	Oat Flavor	Sweet Flavor	Objectionable Flavor
0	4.97±0.16	4.51±0.11	0.00±0.00	4.17±0.10	4.35±0.13	4.17±0.10	4.31±0.07	0.11±0.01
30	4.50±0.18	4.04±0.14	0.33±0.07	3.62±0.12	3.79±0.26	3.62±0.32	3.71±0.06	0.50±0.21
60	5.19±0.08	4.09±0.21	0.00±0.00	3.52±0.08	3.90±0.16	3.81±0.08	4.47±0.08	0.19±0.08

The ANOVA presents significant difference ($p < 0.05$) by the effect of time in all the response variables. The changes in sweet flavor, crunch and odor characteristic were more drastic compared with the other attributes, which decrease the values when the storage time increases. However the rating between 30 and 60 days are similar, determining that the sensory lost is more appreciable between 0 and 30 days.

In any of this three study times the odor was not objectionable, this could be due for what is explain by Cac eres *et al.*, 2008 (33) in a study performed with sausages with ω -3, where this attribute is appreciable when the levels of fat are low. It determines that the fat plays an important role when this component is used because it masks the odor and flavor of ω -3.

CONCLUSIONS

With this study was found that the emulsifiers properties of ω -3 (DENOMEGA GAT 100), help to the incorporation of air to the system and the softness of the cookies. The liquid state provides hardness conditions undesirable for the texture of the product and the dispersion of the data always was high compared with the emulsion, determining that the product tends to be unstable. The ω -3 powder influences the rheology of the dough, taking tenacity to higher values and poor extensibility; besides the ω -3: ω -6 ratios have an incidence in the final product; it is observed that when the values increase, they have a negative effect in each variable response evaluated. The process at 160 C - 7 minutes decreases the moisture which is directly related with the shelf life of the product, and cookies fracturability increases, which

make the product crunchier compared with the process at 150°C - 8 minutes. After all the study is determined that better combination is the emulsion type and 1:10 ratio, because its better results in dough and cookie performance, besides during the storage time has good stability. The losses of DHA + EPA was approximately 6%.

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