Grasas y Aceites 76 (3)
July-September 2025, 2291
ISSN-L: 0017-3495, eISSN: 1988-4214
https://doi.org/10.3989/gya.0212251.2291

Evaluation of rancidity and sensory response of butter added with oregano (*Origanum vulgare*), cloves (*Syzygium aromaticum*), and chili (*Capsicum annum*)

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Submitted: 19 February 2025; Accepted: 15 September 2025; Published: 06 March 2026.

SUMMARY: This study evaluates the addition of 1% oregano, cloves, and chili pepper to butter. The spices are analyzed and the high dietary fiber and ash contents are highlighted. Butter acidity increases with storage time in all samples, being significant in butter with oregano and cloves ($p < 0.05$), while adding chili pepper does not modify the acidity kinetics compared to the control butter. From the sensory point of view, all samples presented high acceptability, with butter with chili pepper standing out as being significantly ($p < 0.05$) better rated in appearance and color; it was also more preferred. Based on the conditions and results of this study, chili pepper was selected to be added to butter at 1%.

KEY-WORDS: *Butter; Chili pepper; Cloves; Oregano; Rancidity; Sensory.*

RESUMEN: ***Evaluación de la rancidez y la respuesta sensorial de la mantequilla adicionada con orégano* (Origanum vulgare), *clavo de olor* (Syzygium aromaticum) *y chile* (Capsicum annum).** Este estudio evalúa la adición de 1% de orégano, clavos de olor y ají en mantequilla. El análisis de especias destaca el alto contenido de fibra dietética y cenizas. La acidez de mantequilla aumenta en el tiempo de almacenamiento en todas las muestras, siendo significativo en mantequilla con orégano y clavos de olor ($p < 0,05$), mientras que la adición de ají no modifica la cinética de acidez comparada con mantequilla control. Desde el punto de vista sensorial, todas las muestras presentaron alta aceptabilidad, destacándose mantequilla con ají que fue significativamente ($p < 0,05$) mejor calificada en apariencia y color; también tuvo mayor preferencia. En base a las condiciones y resultados del presente estudio, se selecciona el ají para adicionar a mantequilla al 1%.

PALABRAS CLAVE: *Ají; Clavos de olor; Mantequilla; Orégano; Rancidez; Sensorial.*

Citation/Cómo citar este artículo: Quitral V, Armijo E, Guzmán B, Flores M, Sepúlveda M. 2025. Evaluation of rancidity and sensory response of butter added with oregano (*Origanum vulgare*), cloves (*Syzygium aromaticum*), and chili (*Capsicum annum*). *Grasas Aceites* **76** (3), 2291. https://doi.org/10.3989/gya.0212251.2291

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1. INTRODUCTION

Butter is a water-in-oil emulsion containing almost 100% milk fats, with triglycerides being its major constituent. Its composition is complex; more than 400 fatty acids have been identified, with a predominance of saturated fatty acids. Among the saturated acids, palmitic (C16:0) predominates, followed by stearic (C18:0), lauric (C12:0), and myristic (C14:0) (Karakus *et al*., 2022).

Due to its high fat content, butter is susceptible to hydrolytic rancidity. Hydrolytic rancidity is influenced by the presence of water, and butter contain water because it is a W/O emulsion, so breaks down fats into free fatty acids and glycerol. Other influences are catalysts with enzymes (lipases), acids, or bases. When triglycerides are hydrolyzed, fatty acids are released, causing strange, rancid aromas and flavors which are easily detected and affect the shelf life of the food. These are determined by free acidity analysis. The "butyric" aroma of rancid butter is characteristic, primarily due to the release of butyric acid, a short-chain and volatile compound, which is why it is sensory perceived in rancid butter (Danudol and Judprasong, 2022). Some studies have reported on the effect of butyric acid on the inhibition of cancer cell growth and the reduction of the risk of atherosclerosis. Therefore, butter has a healthier value than other fats (Fei *et al*., 2017; Peter, 1997).

Antioxidants delay the rancidity phenomena, although synthetic antioxidants have been questioned (Wang *et al.,* 2021), while the use of natural additives is a current trend, leaning towards healthier and more sustainable alternatives, such as herbs and spices (Cerecedo-Cruz *et al*., 2018; Dupas *et al*., 2020; Serra *et al*., 2024), either using them directly, as extracts or essential oils.

Since ancient times, many spices have been used in food for preservation, thanks to their antioxidant and antimicrobial effects, and also contributed to their anti-inflammatory and pharmacological effect. On the other hand, they increase the aromas and flavors of food (Serra *et al*., 2024; Dupas *et al*., 2020; Xu *et al*., 2023).

The leaves of dried oregano (*Origanum vulgare*), just like its extract, have served as antioxidants in different food matrices. Polar compounds have been isolated from its leaves: rosemary acid, caffeic acid, protocatechuic acid, and derivatives. The essential oils obtained from oregano contain carvacrol and thymol (Ferreira-Anta *et al*., 2024; Pokorny *et al*., 2005), despite their antibacterial, antifungal, and antioxidant properties, these compounds have high volatility, low stability, and hydrophobicity, which can limit their applications.

Cloves (*Syzygium aromaticum*) are characterized by its special aroma, in addition to healthy and pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, antifungal, and antiviral. The main compound in cloves is eugenol, which corresponds to a volatile phenylpropanoid (Ramadan *et al*., 2013; Frohlich *et al*., 2023). Clove oil contains more polyunsaturated fatty acids, highlighting linoleic acid. Its content of tocopherols and tocotrienols stands out, and contributes to the stability of the oil against oxidation (Ramadan *et al*., 2013).

Chili pepper (*Capsicum annum*) has a pungent aroma and flavor that makes it very attractive to incorporate into foods. It contains bioactive compounds such as capsaicinoids, carotenoids, phenols, flavonoids, and others (Gamarra 2022; Loizzo *et al*., 2015). These spices provide aromas and flavors when incorporated into foods, such as butter, and can also delay rancidity due to their bioactive components. The antioxidant power of smoked chili comes from phenolic compounds, capsaicinoids, and carotenoids, and the hydrophilic fraction of these compounds is the main contributor to the antioxidant activity of chili (Cerecedo-Cruz *et al*., 2018).

This study aimed to evaluate the hydrolytic rancidity over time of butter with added spices and compare the sensory response to their consumption.

2. MATERIALS AND METHODS

2.1. Materials

Commercial butter made exclusively from cream with 15.5 g/100 g of moisture was purchased. Spices were purchased in powder, ground, and packaged form.

2.2. Sample preparation

1% of each spice was added to butter samples, obtaining samples M1, M2, and M3 with added oregano, cloves, and chili pepper, respectively. For this purpose, the butter samples were gently shaken until they were homogeneous, then they were refrigerated at 5 °C. A control sample was kept without the addition of spices (M4).

2.3. Chemical analysis of samples

2.3.1. Proximate analysis of spices

The spice samples were analyzed using official methods (A.O.A.C., 2005). Moisture was determined using the thermogravimetric method of drying in an oven at 105 °C until constant weight. Proteins were determined using the Kjeldahl method. Total fat was determined using the Soxhlet solvent extraction method with petroleum ether. Ash was determined using the thermogravimetric calcination method at 550 °C. Dietary fiber was determined using the gravimetric enzymatic method applying the enzymes alpha-amylase, protease and amyloglucosidase (A.O.A.C., 2005), then the residue was filtered and weighed. Carbohydrates were determined by calculating the difference [100-(moisture + ash + proteins + fat + dietary fiber)].

The analyses were performed in duplicate. The results were analyzed using an analysis of variance (ANOVA) with post-hoc Tukey's test to establish significant differences ($\alpha = 5\%$). The SPSS program was used.

2.3.2. Acidity of spices

To determine the acidity of the spices, approximately 25 g were weighed with a precision of 0.0001 g, transferred to an-Erlenmeyer flask with 50 ml of boiled and cooled distilled water, stirring to homogenize. The mixture was heated to boiling for 30 minutes, cooled, and transferred to a 250-ml volumetric flask, topped up with distilled water. It was mixed well and filtered. A 10 ml aliquot of the filtrate was taken and titrated with a 0.1 N NaOH solution in the presence of phenolphthalein as an indicator until it turned a light pink color (A.O.A.C., 2005). The acidity was expressed as % of citric acid, applying the following equation:

$$Acidity\ (\%\ Citric\ acid) = \frac{V\ x\ N\ x\ 64}{m}$$

V = Volume of base used in titration (ml)
N = normality of NaOH
64 = factor for citric acid
m = mass of sample (g)

The analyses were performed in duplicate. The results were subjected to an analysis of variance (ANOVA) with post-hoc Tukey's test to establish significant differences ($\alpha = 5\%$). The SPSS program was used.

2.3.3. Acidity of butter

A titration method was applied. Approximately 10 g of a representative sample of each butter was weighed in an Erlenmeyer flask with a precision of 0.0001 g. 50 ml of ethanol were added to dissolve the fat, stirring manually, and the flasks were transferred to a thermoregulated bath with stirring for 30 minutes. The titration was carried out in a 0.1 N NaOH solution using phenolphthalein solution as an indicator until a light pink color (A.O.C.S. 2009). The acidity was expressed as lactic acid using the following formula:

$$Acidity\ (\%\ Lactic\ acid) = \frac{V\ x\ N\ x\ 90}{m}$$

V = Volume of base used in titration (ml)
N = normality of NaOH
90 = factor for lactic acid
m = mass of the sample (g)

The analyses were performed in duplicate. The acidity of the butters was determined for 130 days. Analysis of variance (ANOVA) and post-hoc Tukey's test were applied to establish significant differences ($\alpha = 5\%$) among the samples using the SPSS program.

2.4. Sensory analysis

2.4.1. Acceptability

For sensory acceptability evaluation, a 9-point hedonic scale was applied for each parameter (appearance, color, aroma, flavor, and texture). A response sheet was given with descriptions of the sensation in front of each sample, which was then assigned a numerical value from 1 to 9, with 1 = "I dislike it very much" and 9 = "I love it". 60 consumers participated, healthy men and women between 20 and 65 years old. Each sample was evaluated separately on different days and unsalted and sugar-free biscuits were presented as a vehicle for evaluation (Feng *et al*., 2015).

The results were subjected to an analysis of variance (ANOVA) with Tukey's test to establish significant differences ($\alpha = 5\%$) among the samples. The program was used to work with SPSS.

2.4.2. Preference

Sensory preference was assessed using a ranking test (UNE-ISO Standard, 2010) with 60 healthy male and female consumers aged between 20 and 65 years old. The 3 spiced butter samples were presented, each labeled with 3 random digits, and unsalted and sugar-free biscuits as the vehicle for their assessment. Each subject was asked to rank the samples in 1st, 2nd, and 3rd place according to their preference. The data were analyzed using the Friedman statistic to establish significant differences among the samples with a significance level of 5%.

2.4.3. Focus group

Two focus groups were conducted with 6 participants each. The first group consisted of men and women between 18 and 29 years old, while the second group included subjects between 30 and 50 years old. In both focus groups, the participants' opinions regarding the butter samples were explored, inquiring about the characteristics that they liked or disliked, as well as the consumption occasions that they would consider for each sample (Andres, 2013). The results obtained that correspond to the opinions of the participants are ordered by categorization.

3. RESULTS AND DISCUSSION

3.1. Proximal analisis in spices

From Table 1, it can be deduced that moisture is low in all three spices, and significant differences ($p < 0.05$) are found among the samples. Protein content m.s. is significantly higher in chili pepper and total fat m.s. in cloves. The fat content is high in the three spices due to their essential oil content; in addition, they are dehydrated, which causes their components to become concentrated. The carbohydrate content is high, especially in cloves. Generally, this component, along with dietary fiber, is abundant in spices. Dietary fiber is high in all three spices, with no significant differences. Ash content is high in all three spices and significantly ($p < 0.05$) higher in chili pepper. This is because spices contain many minerals. The caloric intake is high in all three spices and is significantly higher ($p < 0.05$) in cloves.

TABLE 1. Proximate analyses of spices

Parameter	Simples of spices		
	Oregano (*Origanum vulgare*)	Cloves (*Syzygium aromaticum*)	Chili pepper (*Capsicum annum*)
Moisture (g/100 g)	6.1 ± 0.1^{b}	7.8 ± 0.2^{c}	5.0 ± 0.1^{a}
Proteins (g/100 g d.w.)	10.0 ± 0.6^{ab}	6.0 ± 1.1^{a}	12.6 ± 1.6^{b}
Fat (g/100 g d.w.)	4.8 ± 0.3^{a}	17.0 ± 1.4^{b}	5.0 ± 0.3^{a}
Carbohydrates (g/100 g d.w.)	30.5 ± 4.9^{ab}	35.0 ± 0.1^{b}	22.2 ± 1.0^{a}
Dietary fiber (g/100 g d.w.)	42.7 ± 3.7^{a}	35.0 ± 0.5^{a}	34.8 ± 3.9^{a}
Ash (g/100 g d.w.)	12.0 ± 0.9^{b}	7.0 ± 0.7^{a}	25.4 ± 1.0^{c}
Energy (kcal/100 g d.w.)	193 ± 18.3^{a}	292 ± 7.4^{b}	175 ± 12.5^{a}

Values are mean ± standard deviation (n = 2). Different letters in rows indicate significant differences (p < 0.05) as analyzed by Anova and Tukey's test). D.w.: dry weight

The values determined in this study are like those reported in food composition tables and other research (BEDCA, 2025; INTA-UChile, 2018; Al Dhaheri *et al*., 2023). However, the composition can vary depending on the type of soil they are grown in, the geographical area of production, as well as the degree of milling and storage conditions (Al Dhaheri *et al*., 2023).

3.2. Acidity of spices

It can be seen from Table 2 that there are significant differences (p < 0.05) in the acidity of the samples, being higher in cloves.

TABLE 2. Acidity of spice samples expressed as citric acid

Spice samples		
Oregano (*Origanum vulgare*)	Cloves (*Syzygium aromaticum*)	Chili pepper (*Capsicum annum*)
1.25 ± 0.03^{b}	1.83 ± 0.01^{c}	0.80 ± 0.02^{a}

Values are mean ± standard deviation (n = 2). Different letters in rows indicate significant differences (p < 0.05) as analyzed by Anova and Tukey's test.

Kodavatiganti (2021) reported that cloves are more acidic in a group of 17 spices analyzed. The acidity determined in the present study for chili pepper exceeds the values presented by Jiménez *et al*. (2013), which vary from 0.14 to 0.43% citric acid, and exceed the values of the study by Gapasín and Salas (2023).

3.3. Butter acidity

From Figure 1, it can be seen that acidity increases during storage time, and then decreases after 130 days.

There are no significant differences between M3 and M4 during the 130 days. However, samples M1 and M2 do show significant differences, with acidity being higher in M2. The increase in acidity reflects greater hydrolytic rancidity, which reduces shelf life as it causes strange, altered, and unpleasant aromas and flavors. Erkaya *et al*. (2015) report higher values than those determined in the present study, with 0.35 to 0.42% lactic acid in butter.

The addition of 1% chili pepper to butter appears to be a feasible alternative since the acidity is similar to that of the control sample. The addition of 1% oregano and cloves to butter increases rancidity, which decreases shelf life by more than 90%, making it necessary to add synthetic antioxidants or acidity regulators.

The results obtained can be attributed to the higher moisture content and acidity in cloves followed by oregano. The presence of moisture catalyzes the hydrolysis reaction of triglycerides as well as acidity, which makes these spices not suitable for adding to butter.

Damechki *et al*. (2001) added 5% oregano to olive oil and managed to improve the sensory characteristics compared to the control sample; the polyphenol content increased significantly, and α-tocopherol decreased; In addition, the peroxide index increased, as did the chlorophyll content.

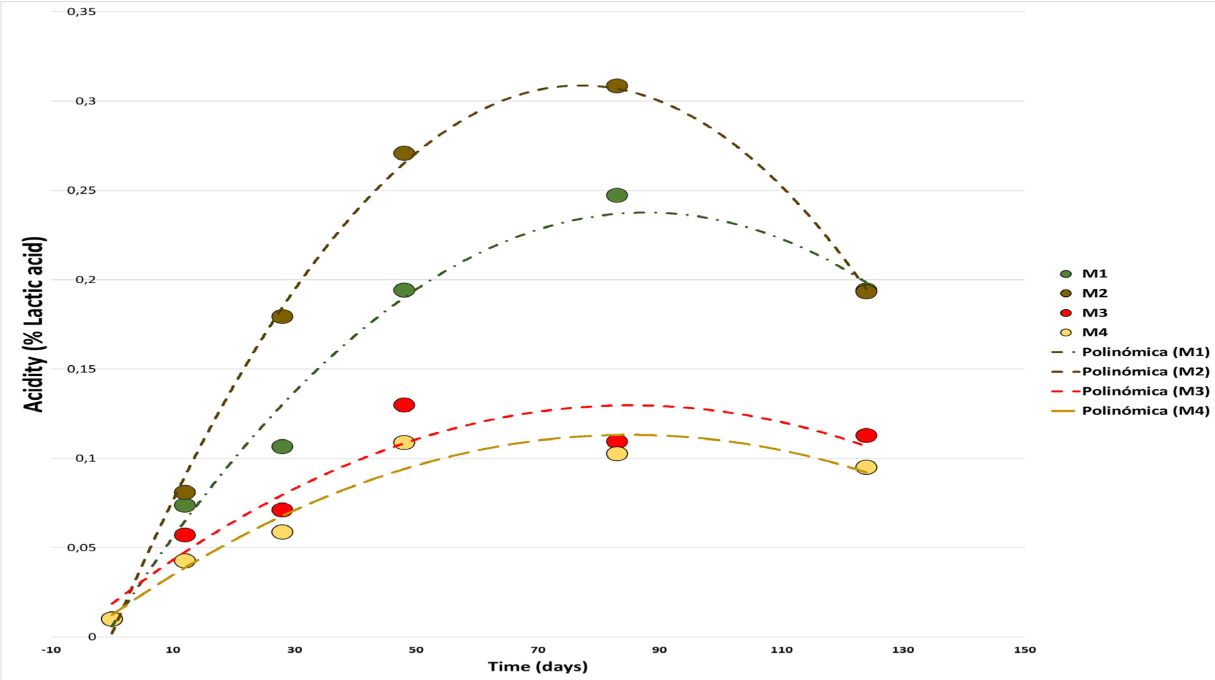

FIGURE 1. Titrable acidity of butter samples over time. M1 = butter + 1% oregano. M2 = butter + 1% clove. M3 = butter + 1% chili pepper. M4 = butter control.

Herbs and spices are rich in phenolic compounds and others with high antioxidant capacity, so many studies demonstrate their protective effect against rancidity in fatty materials (Farag *et al*., 1990; Augusto-Obara *et al*., 2017; Embuscado, 2015), but there are also cases in which some herbs or spices act as prooxidants (Ayar *et al.,* 2001; Serra *et al.,* 2024). This paradox could be interpreted by the insolubility in the non-polar media of phenolic compounds. In the case of oregano, another factor to consider is the presence of chlorophyll because there is evidence of a prooxidant effect in oils (Damechki *et al.,* 2001).

Najgebauer-Lejko *et al*. (2009) investigated the addition of herbs to butter and concluded that they generally do not prevent lipolysis and peroxide formation during storage, the authors attribute this to the microbiological instability of herbs and spices.

3.4. Sensory acceptability

Figure 2 shows no significant differences among the samples for the texture, flavor, and odor attributes. All samples scored high on the 9-point hedonic scale, indicating high overall acceptability.

As for color and appearance, slight differences were observed. Sample M3 scored the highest, suggesting a greater acceptability, followed by M1. Sample M2 was ranked third for the color attribute, while M1 was third for appearance. Despite these variations, all three samples scored high fof both attributes.

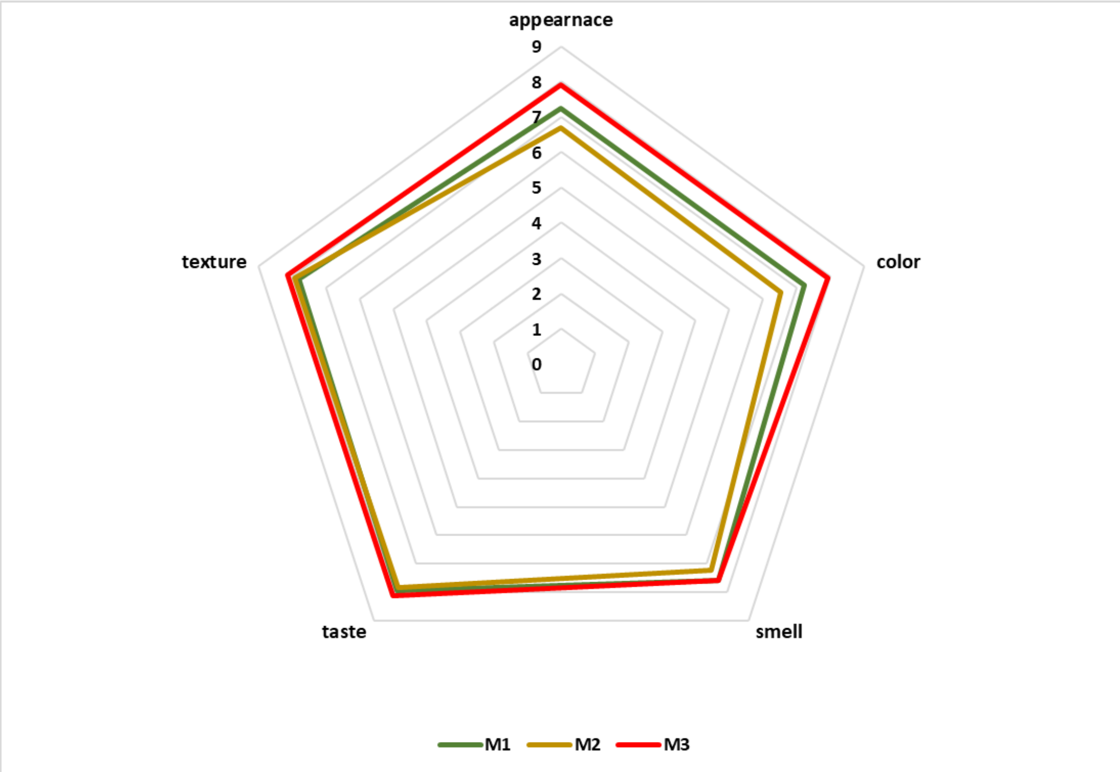

FIGURE 2. Sensory acceptability of butter samples evaluated with a 9-point hedonic scale as analyzed by Anova and Tukey's test. 1 repetition. 60 evaluators. M1 = butter + 1% oregano. M2 = butter + 1% clove. M3 = butter + 1% chili pepper.

3.5. Sensory preference

Although there are no significant differences in the order of preference of the samples, Table 3 shows a trend of greater preference for sample M3.

TABLE 3. Order of sensory preference of butter samples

Preference	Samples
1°	M3[a]
2°	M1[a]
3°	M2[a]

Equal letters in the rows indicate that there are no significant differences, as analyzed by Friedman statistic. 1 repetition, 60 evaluators. M1 = butter + 1% oregano M2 = butter + 1% clove. M3 = butter + 1% chili pepper.

3.6. Focus group

The main comments made in the focus group are presented in Table 4.

TABLE 4. Evaluators' comments in the focus group

Samples	Featured comments	Suggestions
M1	- Delicious and pleasant taste. - Attractive visual appearance.	- Increase the concentration of oregano. - Balance the buttery flavor, which was perceived as dominant.
M2	- Delicious and "innovative" flavor. - Association with sweet and Christmas preparations.	- Reduce the intensity of the clove flavor. - Improve the color, since it is dark and opaque.
M3	- Attractive and "less opaque" color. - Spicy flavor with a pleasant finish. - Association with wine or beer consumption and "paprika cream."	- Increase the concentration of chili to intensify its flavor.

M1 = butter + 1% oregano. M2 = butter + 1% clove. M3 = butter + 1% chili pepper

Considering sensory evaluations, it is noted that the least favored sample corresponds to M2 (butter + 1% cloves), which, although its flavor was indeed attractive and novel, the color is not attractive, and it impairs the sensory response. On the other hand, M1 (butter + 1% oregano) was very pleasant, its color was well evaluated on a hedonic scale (7.2 points). Some evaluators suggested increasing the concentration of oregano, which could be tested in future studies. Sample M3 (butter + 1% chili) had a very attractive color. Some people are familiar with the consumption of chili, so they proposed increasing the concentration. The evaluators in general rated the flavor of M3 very well (8.1 points).

4. CONCLUSIONS

The spices investigated had high contents of dietary fiber and ash, although they were not transferred to the butter samples because they were incorporated at only 1%.

The rancidity of the butter samples with oregano and cloves increased over time, as demonstrated by the acidity kinetics. This was contributed to by the higher moisture content and acidity of these spices, primarily cloves. On the other hand, the addition of 1% chili pepper in butter did not alter the rancidity of the butter, which remained similar to the control sample over time.

Considering the sensory response of acceptability, preference and focus group, the butter sample with 1% chili pepper was the outstanding option. It did not increase the rancidity of the butter either.

Research is expected to continue with the addition of different concentrations of oregano and chili pepper in butter and a control of rancidity.

ACKNOWLEDGMENTS

The authors are thankful to the Escuela de Nutrición y Dietética, Universidad Santo Tomás for providing the necessary resources.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

FUNDING SOURCES

This study received no external funding.

AUTHORSHIP CONTRIBUTION STATEMENT

VQ, MF, MS: Conceptualization, Investigation. MF, VQ, EA, BG: Data curation, Methodology, VQ, MF: Formal analysis. VQ: Project administration. VQ, MS: Resources, Supervision, Validation, Writing- original draft.

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