GRASAS Y ACEITES 74 (1)
January-March 2023, e489
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.1122212

Fatty acid profile and rheological behavior of annatto seed oil (*Bixa orellana*), cupuassu seed fat (*Theobroma grandiflorum*), and their blends

D.C.S. da Silva[a], F.B. de Carvalho-Guimarães[b], I.P. Valente[a], N. Cunha[a], S.C. da C. Sanches[b], D.A. da Silva[a], J.O.C. Silva Júnior[b], L.H.M. da Silva[a,✉] and A.M. da C. Rodrigues[a]

[a]Postgraduate Program in Food Science and Technology (PPGCTA), Institute of Technology (ITEC), Federal University of Pará (UFPA), 66075-110, Belém, Pará, Brazil.

[b]Postgraduate Program in Pharmaceutical Innovation (PPGIF), Institute of Health Sciences (ICS), Federal University of Pará (UFPA), 66075-110, Belém, Pará, Brazil.
✉Corresponding author: lhmeller@ufpa.br

Submitted: 08 November 2021; Accepted: 15 March 2022; Published online: 22 March 2023

SUMMARY: Annatto seed oil (ASO) and cupuassu seed fat (CSF) were combined at the ratios: 30:70, 50:50, and 70:30 (% w/w). Their fatty acid profile, nutritional quality, FTIR (Fourier Transform Infrared) spectra, and rheological behavior were evaluated. ASO increased the content of polyunsaturated fatty acids in the blends; whereas CSF conferred higher contents of monounsaturated fatty acids. The blends exhibited low atherogenicity and thrombogenicity indices, suggesting nutritional advantages. The Newtonian fluid behavior and FTIR results suggested that mixing ASO and CSF at different proportions did not affect the functional groups. ASO showed an activation energy value which indicated that this fat viscosity was more sensitive to temperature changes. The Newtonian model proved to be suitable to describe the behavior of samples, according to statistical fit parameters R^2, χ^2, and RSS. The resulting blends presented improved physicochemical properties and nutritional attributes, indicating their feasibility for the development of new products.

KEYWORDS: *Activation energy; Amazonian matrices; Nutritional quality; Viscosity.*

RESUMEN: ***Perfil de ácidos grasos y comportamiento reológico de aceite y grasa de semillas de achiote (*****Bixa orellana*****), y de cacao blanco (*****Theobroma grandiflorum*****) y sus mezclas.*** Se combinaron aceite de semilla de achiote (ASO) y grasa de semilla de cacao blanco (CSF) en las proporciones: 30:70, 50:50 y 70:30 (% p/p), respectivamente. Se evaluó su perfil de ácidos grasos, calidad nutricional, espectros FTIR (Fourier Transform Infrared) y comportamiento reológico. ASO incrementó el contenido de ácidos grasos poliinsaturados en las mezclas, mientras que CSF confirió mayores contenidos de ácidos grasos monoinsaturados. Las mezclas exhibieron bajos índices de aterogenicidad y trombogenicidad, lo que sugiere ventajas nutricionales. El comportamiento del fluido newtoniano y los resultados de FTIR sugirieron que mezclar ASO y CSF en diferentes proporciones no afectó a los grupos funcionales. ASO mostró un valor de energía de activación que indicó que la viscosidad de esta grasa era más sensible a los cambios de temperatura. El modelo newtoniano demostró ser adecuado para describir el comportamiento de las muestras, según los parámetros de ajuste estadístico R^2, χ^2 y RSS. Las mezclas resultantes presentaron propiedades fisicoquímicas y atributos nutricionales mejorados, lo que indica su viabilidad para el desarrollo de nuevos productos.

PALABRAS CLAVE: *Calidad nutricional; Energía de activación; Matrices amazónicas; Viscosidad.*

Citation/Cómo citar este artículo: da Silva DCS, de Carvalho-Guimarães FB, Valente IP, Cunha N, da C. Sanches SC, da Silva DA, Silva Júnior JOC, da Silva LHM, da C. Rodrigues AM. 2023. Fatty acid profile and rheological behavior of annatto seed oil (*Bixa orellana*), cupuassu seed fat (*Theobroma grandiflorum*), and their blends. *Grasas Aceites* **74** (1), e489. https://doi.org/10.3989/gya.1122212

1. INTRODUCTION

The current demand for oils and fats, with emphasis on healthy foods and new uses, has accelerated the search for alternative sources of these lipids. However, many oils and fats with interesting characteristics and unique compositions have not yet been fully considered for product formulation due to the scarcity of information about their properties, as is the case of some Amazonian matrices (Serra *et al.,* 2019).

Bixa orellana L., also known as annatto, is a species native to tropical America. In Brazil, it is native to the North and Northeast regions and occurs mainly in the states of Amazonas, Pará, Paraíba, Piauí, Maranhão, Ceará, and Bahia (Stringheta *et al*., 2018). Annatto is an important source of natural dyes, such as fat-soluble bixin and water-soluble norbixin, with colorations ranging from yellow to dark red. These dyes are widely used in the food, pharmaceutical, and cosmetic industries, due to their high stability, coloring capacity, and lack of toxicity. In addition, the oil extracted from annatto seeds has been arousing the interest of many industrial sectors due to the presence of δ-tocotrienol (Costa *et al*., 2013), which are antioxidant compounds used in the treatment of cardiovascular diseases and cancer, and are also effective in preventing lipid oxidation (Costa *et al*., 2013; Shen *et al.,* 2021).

Cupuassu (*Theobroma grandiflorum*) is a fruit which is native to the Brazilian Amazon and has high agro-economic potential. Its pulp presents wide acceptance in the national and international markets for its unique characteristics such as flavor, aroma, and texture. It is also worth mentioning that a natural fat with chemical and sensory characteristics similar to those of cocoa butter is obtained from cupuassu seeds (Bezerra *et al*., 2017). Cupuassu fat has been considered an excellent source of monounsaturated fatty acids (MUFA) because it has a great content of oleic acid in its composition. These fatty acids have greater oxidation stability when compared to polyunsaturated fatty acids (PUFA), in addition to reducing the levels of LDL cholesterol and lowering the risk of coronary heart disease (Bezerra *et al*., 2017; Serra *et al*., 2019).

Knowledge of the composition and physicochemical properties of oils and fats and their blends is essential for product development, as well as for designing and improving industrial processes. Iodine value (IV) measures the degree of unsaturation of fatty acids present in oils and fats. Since the melting point and oxidative stability of oils and fats are related to the degree of unsaturation, IV can provide an estimation of these properties. The higher this value, the higher the degree of unsaturation and the greater the susceptibility to oxidation (Dymińska *et al.*, 2016).

Nutritional quality indexes are often used to evaluate oils and fats because they allow a better understanding of the health effects of fatty acids (Attia *et al*., 2015). Atherogenicity index (AI) and thrombogenicity index (TI) are directly related to the stimulation of platelet aggregation (Ulbricht and Southgate, 1991). Therefore, lipids with lower AI and TI values have greater potential to prevent coronary heart diseases.

The most important factors for the utilization of oilseed matrices in industries are quality, stability, and nutritional and functional characteristics of the oils and fats obtained. Although pure oil (as example, palm, sesame, canola, soy and olive oil) is not suitable for direct use in the development of new products (Hashempour-Baltork *et al.,* 2016), blends of oils and fats have been widely applied, due to changes in their natural physicochemical characteristics. Thus, by simply combining different matrices, it is possible to obtain products with interesting technological, nutritional, and functional properties (Bezerra *et al.,* 2017).

In this context, considering the little information available and the increasing demand for new alternative lipid sources, the present study aimed to obtain blends of different proportions of annatto seed oil (ASO) and cupuassu seed fat (CSF), as well as to determine their fatty acid profiles, nutritional quality indices, Fourier Transform Infrared (FTIR) spectra, and rheological behavior.

2. MATERIALS AND METHODS

2.1. Materials and blend preparation

The ASO sample was provided by *Gran Oils* Industry (São Paulo, Brazil) and The CSF sample was provided by *Amazon Oil* Industry (Ananindeua, Pará, Brazil). For blend preparation, ASO and CSF samples were previously heated at 40 ºC, in order to facilitate the homogenization process.

2.2. Determination of fatty acid composition

The fatty acid profiles of ASO, CSF, and their blends were determined by gas chromatography (GC) (CP 3380, Varian Inc., USA) equipped with a flame ionization detector (FID) and a CP-Sil 88 capillary column (60 m x 0.25 mm x 0.25 mm) (Varian Inc., USA). Fatty acid methyl esters were prepared based on the method proposed by Rodrigues *et al.* (2010) and the operating conditions were helium as carrier gas at a flow rate of 0.9 mL·min^{-1}, FID at 250 °C, and injector split ratio of 1:100 at 245 °C. The column temperature was programmed at 80 ºC for 4 min, with a subsequent increase to 220 ºC at 4 ºC·min^{-1}. Individual fatty acid peaks were identified by comparison of retention times of the gas chromatography with those of known mixtures of fatty acid standards (74X Nu-check-prep, Inc., USA) run under the same operating conditions.

2.3. Iodine and saponification values

Iodine and saponification values were obtained according to methods Cd 1c-85 and Cd 3-94 (AOCS, 2004), respectively, based on the fatty acid compositions.

2.4. Lipid quality indexes

The nutritional quality of ASO, CSF, and their blends was evaluated using six nutritional indicators based on fatty acid compositions. Atherogenicity (*AI*) and thrombogenicity (*TI*) indexes and the ratio of polyunsaturated to saturated fatty acids (*P/S*) were determined according to Ulbricht and Southgate (1991) using Equations 1, 2, and 3, respectively. The ratio of hypocholesterolemic/hypercholesterolemic fatty acids (*h/H*) was determined according to Santos-Silva *et al.* (2002), using Equation 4. And the nutritive value index (*NVI*) was determined according to Chen *et al.* (2016), using Equation 5.

$$\mathrm{AI} = \frac{[\mathrm{C12:0} + 4 \times (\mathrm{C14:0}) + \mathrm{C16:0}]}{[\sum \omega 6 + \sum \omega 3 + \sum \mathrm{MUFA}]} \quad (1)$$

$$\mathrm{TI} = \frac{(\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0})}{[(0.5 \times \sum \mathrm{MUFA}) + (0.5 \times \sum \omega 6) + 3 \times \sum \omega 3) + (\sum \omega 3 / \sum \omega 6)]} \quad (2)$$

$$\mathrm{P/S} = \frac{\sum \mathrm{PUFA}}{\sum \mathrm{SFA}} \quad (3)$$

$$NVI = \frac{(C18{:}0 + C18{:}1)}{C16{:}0} \quad (5)$$

2.5. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

The spectra of ASO, CSF, and their blends were obtained with a Fourier transform infrared spectrophotometer (Shimadzu Corporation IR Prestige 21 Cat. No. 206-73600-36- Kyoto-Japan), at the 4000 to 600 cm^{-1} regions, using 32 scans with spectral resolution of 4 cm^{-1}. *OriginPro* v8.0 software was used to graphically plot the spectra obtained, with the aim of evaluating possible changes in functional groups.

2.6. Rheological measurements

The flow behaviors of ASO, CSF, and their blends were determined using a programmable rotational viscometer (DV-II+, Brookfield Engineering Laboratories, USA) equipped with a DIN-87 spindle. The viscometer was coupled to an ultra-thermostatic bath (SL 152/10, Solab, Brazil) for temperature control. Measurements were performed at temperatures of 30, 40, and 50 ºC, with shear rates ranging from 26 to 155 s^{-1}. Shear stress (τ) and viscosity (η) were obtained using *WinGather* software (version 1.1, Brookfield Engineering Laboratories, USA).

2.6.1. Rheological modeling

Oils and fats generally exhibit a linear relationship between shear stress and strain rate, and the Newtonian model is the most commonly used to describe their flow behaviors. Therefore, the data obtained experimentally were fitted to the Newtonian rheological model (Equation 6).

$$\tau = \eta \dot{\gamma} \quad (6)$$

Where τ = shear stress (Pa); η = viscosity (Pa.s); $\dot{\gamma}$ = shear rate (s^{-1}).

The fitting of data to the Newtonian model was performed by nonlinear regression analysis using

$$h/H = \frac{C18{:}\omega 91 \;\; + C18{:}2\ \omega 6 + C20{:}4\ \omega 6 + C18{:}3\ \omega 3 + C20{:}5\ \omega 3 + C22{:}5\ \omega 3 + C22{:}6\ \omega 3}{C14{:}0 + C16{:}0} \quad (4)$$

OriginPro software (version 8.0, OriginLab, USA). The coefficient of determination (R^2), the reduced chi-square value (χ^2), and the residual sum of squares (RSS) were the parameters used to evaluate the fits.

2.6.2. Influence of temperature on rheological behavior

The Arrhenius equation (Equation 7) was used to evaluate the influence of temperature on the viscosity (η) of the samples. The fitting of data to the Arrhenius model was performed by nonlinear regression analysis using *OriginPro* software (version 8.0, OriginLab, USA).

$$ln\,\eta = ln\,A + \frac{E_a}{RT} \quad (7)$$

Where η = viscosity (mPa.s); A = pre-exponential factor (Pa.s); E_a = activation energy (kJ mol^{-1}); R = universal gas constant (8.314 J/mol K); T = absolute temperature (K).

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition, iodine value, and saponification value

The fatty acid composition, iodine and saponification values, and nutritional quality indexes of ASO, CSF, and their blends are presented in Table 1. The results indicate that ASO is rich in *PUFA* (49.25%) and *MUFA* (31.02%), whereas CSF presents high contents of saturated (42.56%) *SFA* and *MUFA* (40.60%). The main fatty acids present in ASO were α-linolenic (49.25%), oleic (31.02%), and palmitic (12.73%). Silva *et al.* (2008) obtained similar contents for oleic (33.9%) and palmitic (16.4%) acids using this same method of analysis. However, in their work, linoleic (34.3%) was the main *PUFA* in ASO.

Some differences were also observed in the fatty acid composition of ASO described by Rao *et al.* (2015). They obtained lower contents of oleic (17.5%) and linolenic (15.1%) acids, and greater concentrations of palmitic (26.9%) and stearic (10.8%) acids. Costa *et al.* (2013) suggest that the variations in the fatty acid profile of ASO can be attributed to the diversity of the species studied.

Regarding CSF, the main fatty acids present were oleic (40.60%), stearic (31.78%), α-linolenic (11.56%), and palmitic (7.51%). Previous studies confirm that oleic acid is the major compound found in CSF (41.60-43.17%), followed by stearic (31.60-33.91%), α-linolenic (11.37-11.50%), and palmitic (7.37-7.50%) acids (Bezerra *et al.,* 2017; Serra *et al.,* 2019). The high content of oleic acid characterizes CSF as a good source of *MUFA*. Bhattacharjee *et al.* (2020) highlighted the role of oleic acid in reducing the risk of cardiovascular diseases, decreasing inflammatory responses, and regulating serum cholesterol levels. It also has the advantage of having greater oxidative stability compared to *PUFA*.

The main fatty acids present in the blends were oleic (33.89-37.71%), α-linolenic (22.90-38.02%), stearic (14.27-24.28%), and palmitic (9.08-11.16%). Increasing the proportion of ASO in the blends promoted an increase in the content of *PUFA* and a consequent reduction in *SFA*. On the other hand, the samples with greater amounts of CSF also presented higher contents of *MUFA*, which makes the blends more interesting to human nutrition due to the beneficial effects of these fatty acids on the prevention of cardiovascular diseases, besides conferring greater stability (Bezerra *et al.,* 2017).

As expected, ASO showed a higher iodine value (*IV*) (155.52 g $I_2$100 g^{-1}) than CSF (74.30 g I_2 100 g^{-1}) because it contains a greater content of unsaturated fatty acids. Thus, the use of CSF may have contributed to increasing the oxidative stability of the blends.

The saponification value (*SV*) is considered a measurement of the chain length of all fatty acids present in the oil or fat (Sivakanthan *et al.,* 2019). The *SV* of ASO (193.49 mg KOH g^{-1}) was higher than that of CSF (190.92 mg KOH g^{-1}).

3.2. Nutritional quality indexes

Atherogenicity and thrombogenicity indexes lower than 1.0 and 0.5, respectively, are recommended in terms of human health (Fernandes *et al.,* 2014). ASO and CSF showed the same *AI* result (0.16), which indicates that these samples have high and equivalent contents of anti-thrombogenic fatty acids (Table 1). As expected, CSF showed a *TI* (0.66) higher than ASO (0.12), because it presents a greater content of *SFA*, especially stearic acid (31.78%). In addition, ASO presented a great concentration of α-linolenic acid (49.25%), which is a *PUFA* that reduces platelet aggregation potential. The blends showed *AI* and *TI* lower than the recommended values, which is very

TABLE 1. Fatty acid composition (%), iodine and saponification values, and nutritional quality indices of annatto seed oil (ASO), cupuassu seed fat (CSF), and their respective blends.

Fatty acids	**ASO**	**ASO:CSF (% w/w)**			**CSF**
		70:30	**50:50**	**30:70**	
Lauric acid (C12:0)	nd	0.20 ± 0.00^{d}	0.33 ± 0.00^{c}	0.46 ± 0.01^{b}	0.65 ± 0.02^{a}
Myristic acid (C14:0)	0.12 ± 0.01^{e}	0.17 ± 0.00^{d}	0.20 ± 0.00^{c}	0.23 ± 0.00^{b}	0.28 ± 0.00^{a}
Palmitic Acid (C16:0)	12.73 ± 0.07^{a}	11.16 ± 0.10^{b}	10.12 ± 0.09^{c}	9.08 ± 0.10^{d}	7.51 ± 0.10^{e}
Stearic Acid (C18:0)	6.88 ± 0.17^{e}	14.27 ± 0.01^{d}	19.28 ± 0.01^{c}	24.28 ± 0.02^{b}	31.78 ± 0.05^{a}
Oleic acid (C18:1, ω-9)	31.02 ± 0.10^{e}	33.89 ± 0.14^{d}	35.79 ± 0.12^{c}	37.71 ± 0.17^{b}	40.60 ± 0.23^{a}
Linoleic acid (C18:2, ω-6)	nd	1.58 ± 0.01^{d}	2.64 ± 0.01^{c}	3.70 ± 0.02^{b}	5.28 ± 0.03^{a}
α-Linolenic acid (C18:3, ω-3)	49.25 ± 0.10^{a}	38.02 ± 0.02^{b}	30.46 ± 0.01^{c}	22.90 ± 0.01^{d}	11.56 ± 0.01^{e}
Arachidic acid (C20:0)	nd	0.09 ± 0.00^{d}	0.15 ± 0.00^{c}	0.20 ± 0.01^{b}	0.29 ± 0.01^{a}
Behenic acid (C22:0)	nd	0.62 ± 0.00^{d}	1.03 ± 0.00^{c}	1.44 ± 0.01^{b}	2.05 ± 0.02^{a}
∑Saturated	19.73 ± 0.12^{e}	26.51 ± 0.11^{d}	31.11 ± 0.10^{c}	35.69 ± 0.14^{b}	42.56 ± 0.19^{a}
∑Monounsaturated	31.02 ± 0.10^{e}	33.89 ± 0.14^{d}	35.79 ± 0.12^{c}	37.71 ± 0.17^{b}	40.60 ± 0.23^{a}
∑Polyunsaturated	49.25 ± 0.10^{a}	39.60 ± 0.03^{b}	33.10 ± 0.02^{c}	26.60 ± 0.03^{d}	16.84 ± 0.04^{e}
Iodine value (g I_2·100 g^{-1})	155.52	131.35	115.04	98.74	74.30
Saponification value (mg KOH·g^{-1})	193.49	192.72	192.23	191.71	190.92
Atherogenicity index (AI)	0.16	0.16	0.16	0.16	0.16
Thrombogenicity index (TI)	0.12	0.16	0.24	0.35	0.66
Ratio of polyunsaturated to saturated fatty acids (P/S)	2.50	1.49	1.06	0.75	0.40
Hypocholesterolemic/hypercholesterolemic fatty acids (h/H)	6.25	6.49	6.68	6.91	7.37
Nutritive value index (NVI)	2.98	4.31	5.44	6.83	9.64

Triplicate means in the same row followed by different letters are significantly different by Tukey's test ($p \leq 0.05$). nd - not detected. Nutritional indexes determined by indirect method

desirable from a human health perspective (Wołoszyn *et al.,* 2020).

The *P/S* ratio is frequently used to evaluate the nutritional quality of oils and fats. A balanced intake of polyunsaturated and saturated fatty acids is beneficial for controlling serum cholesterol levels. A *P/S* ratio higher than 0.45 is recommended in human diets for the prevention of coronary heart disease and some chronic illnesses such as cancer. The *P/S* ratios of all samples were consistent with the recommended value, except for CSF (*P/S* = 0.40) and the most favorable result was observed for ASO (2.50). In addition, all blends showed *P/S* results which are beneficial to human health and increasing the proportion of *ASO* improved the nutritional quality of the blends.

The value of the *h/H* ratio (hypocholesterolemic/hypercholesterolemic – *h/H*) ranged from 6.25 to 7.37, with the highest value for CSF and the lowest for CSO. The mixtures presented values within this range, and as the concentration of CSF in the mixture increased, this ratio increased. This relationship responds directly to the characteristics of the studied fats. The higher the value of *h/H*, the greater the relationship with risk factors, contributing to the increase in cholesterol, so this is an important measurement in the characterization of fats and their mixtures. It is important to have a low *h/H* ratio in the diet to reduce negative prothrombotic effects caused by increased n–6 linoleic acid concentration in the diet. Despite the limitations, compared to the *PUFA/MUFA*, the *h/H* ratio may more accurately reflect the effect of the fatty acid composition on cardiovascular diseases (Chen and Liu, 2020). Values as low as 0.21 can be found for algae and close to 11.0 to 15.0 for camelina oil (*Camelina sativa*) (Chen and Liu, 2020).

CSF exhibited a greater nutritional value index (*NVI*) value (9.64) than ASO (2.98), which was due to the greater proportion of stearic (*C18:0*) and oleic (*C18:1*) acids present in CSF. Furthermore, it was also observed that the addition of CSF to the blends directly influenced the *NVI*, which ranged from 4.31 to 6.83.

3.3. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

The spectra obtained in the region of 4,000 to 500 cm^{-1} for ASO, CSF, and their blends are presented in Figure 1. The absorption bands of highest intensity were identified in the region of 2937 to 2843 cm^{-1} and can be attributed to axial deformation vibrations of C-H bonds present in methyl (CH_3) and methylene (CH_2) groups, and double bonds (=C-H).

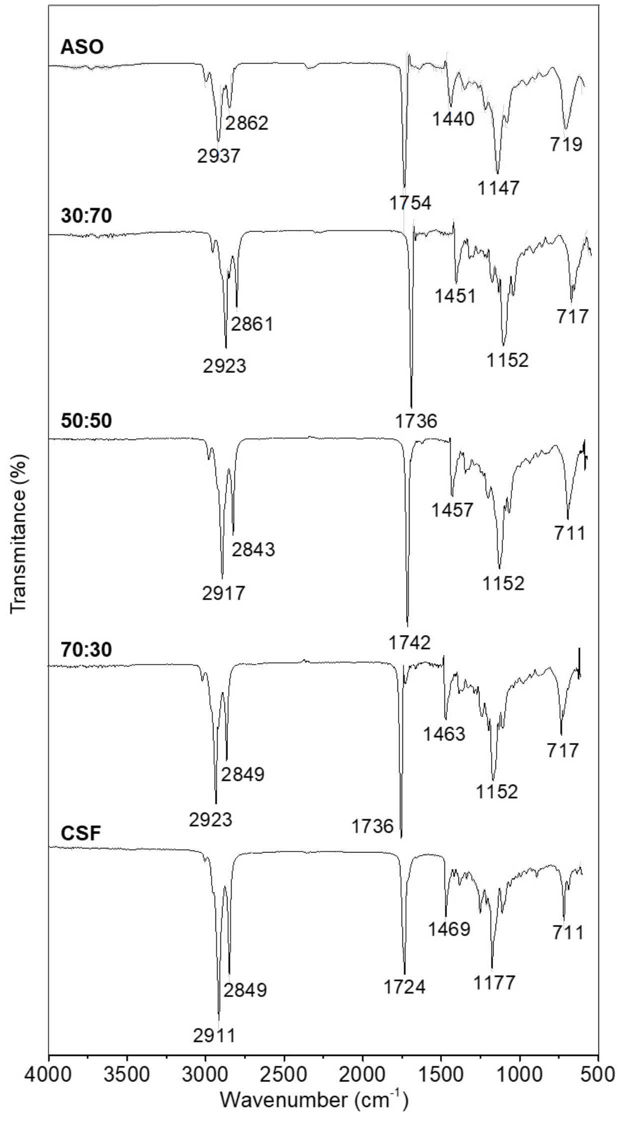

FIGURE 1. Infrared vibrational spectra of annatto seed oil (ASO), cupuassu seed fat (CSF), and their respective blends.

The band that appears in the 1754 - 1724 cm^{-1} region refers to axial deformation vibrations of the carbonyl group (C=O) present in triacylglycerol esters. The bands with intermediate intensity, which appear in the region of 1469 to 1440 cm^{-1}, originate from angular deformation vibrations of C-H bonds present in methyl and methylene groups. The absorption bands which refer to the axial deformation vibrations of C-O bonds of esters which constitute the triacylglycerols are in the 1177 - 1147 cm^{-1} region, which contains part of the "fingerprint" region of the compounds. (García-González *et al.,* 2013). Finally, the peaks at 719 and 711 cm^{-1} can be assigned to the benzene ring.

The results obtained are consistent with the limits reported in other studies (Bitencourt *et al.*, 2018). In addition to the bands identified in this work, a set of absorption bands in the regions of 3338 cm^{-1}, 1612 cm^{-1}, and 1562 cm^{-1} was also found. These data are related to the identification of trans-bixin (carotenoid) present in the oil (Rao *et al.,* 2014).

Regarding the spectra of the blends, it was observed that the vibration bands of the bonds involved are very close and did not undergo relevant shifts with respect to wavelength, indicating the absence of chemical interactions between oil and fat (Silva-Júnior *et al.*, 2008). These results suggest that combining ASO and CSF in different proportions did not affect the functional groups, which is expected since it is just a mixture and there is no reaction.

3.4. Rheological behavior

The rheological curves of shear stress versus shear rate of the pure samples and their blends are presented in Figure 2. All samples showed a linear relationship between shear stress and shear rate, i.e., they exhibited Newtonian fluid behavior (Silva *et al.,* 2017). It can be noted that, for a constant strain rate, there is a decrease in shear stress values with increasing temperature (Figure 2). This effect can be associated with the structural collapse of molecules that constitute the samples, which occurs due to the action of hydrodynamic forces generated, promoting the alignment of molecules (Alparslan and Hayta, 2002).

The Newtonian fluid behavior was confirmed through the parameters of experimental data that were fitted to the Newtonian model, as can be seen in Table 2. R^2, χ^2, and RSS values confirm the excellent fit of data to the Newtonian model, which indicates that this model is able to accurately predict the rhe-

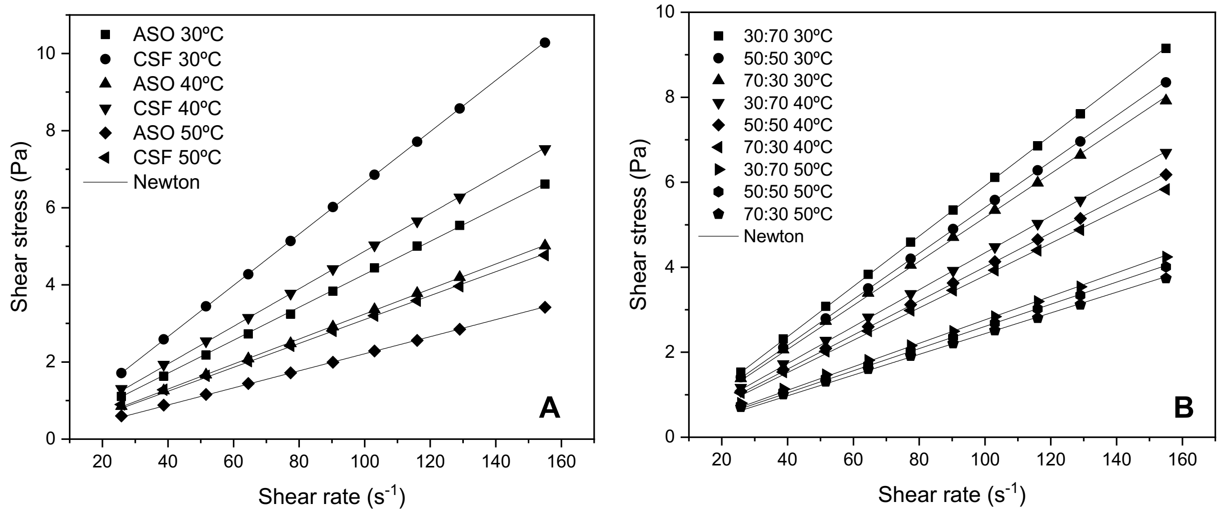

FIGURE 2. Relationship between shear stress and shear rate of pure samples (A) and blends (B), with curves fitted to the Newtonian model.

TABLE 2. Parameters of the Newtonian model fitted to the experimental data of annatto seed oil (ASO), cupuassu seed fat (CSF), and their respective blends.

Sample	T (ºC)	Parameters			
		***η* (Pa s)**	**R^2**	**χ^2**	**RSS**
ASO	30	0.0427^{g}	0.9996^{d}	$0.0013^{c,d}$	0.0118^{g}
	40	0.0324^{j}	$0.9998^{a,b,c}$	0.0003^{e}	0.0026^{m}
	50	0.0221^{o}	$0.9997^{b,c,d}$	0.0003^{e}	0.0023^{n}
ASO:CSF (70:30)	30	0.0517^{d}	0.9993^{e}	0.0031^{a}	0.0281^{a}
	40	0.0380^{i}	0.9990^{f}	$0.0023^{a,b}$	0.0210^{b}
	50	0.0244^{n}	0.9979^{i}	$0.0020^{b,c}$	0.0175^{c}
ASO:CSF (50:50)	30	0.0541^{c}	$0.9999^{a,b}$	0.0003^{e}	0.0028^{l}
	40	0.0401^{h}	$0.9997^{b,c,d}$	$0.0007^{d,e}$	0.0063^{j}
	50	0.0261^{m}	0.9986^{g}	$0.0015^{b,c,d}$	0.0135^{f}
ASO:CSF (30:70)	30	0.0592^{b}	$0.9999^{a,b}$	0.0003^{e}	0.0031^{k}
	40	0.0434^{f}	$0.9997^{c,d}$	$0.0009^{d,e}$	0.0080^{h}
	50	0.0276^{l}	0.9983^{h}	$0.0020^{b,c}$	0.0181^{d}
CSF	30	0.0665^{a}	1.0000^{a}	$0.0002^{d,e}$	0.0015^{o}
	40	0.0487^{e}	$0.9998^{a,b}$	0.0007^{d}	0.0065^{i}
	50	0.0310^{k}	0.9985^{g}	$0.0023^{a,b}$	0.0208^{c}

R^2: Coefficient of determination; χ^2: Reduced Chi-squared; RSS: residual sum of squares. Triplicates mean values with the same letters on the same column do not significantly differ among themselves ($p \leq 0.05$) according to Tukey's test.

ological behavior of ASO, CSF, and their blends, in the temperature range studied.

The Newtonian fluid behavior was confirmed through the parameters of experimental data that were fitted to the Newtonian model because all samples showed high R^2 values (> 0.998) and low χ^2 (< 0.003) and RSS (< 0.028) values, as can be seen in Table 3. R^2, χ^2, and RSS values confirm the excellent fit of data to the Newtonian model, which indicates that this model is able to accurately predict the rheological behavior of ASO, CSF, and their blends, in the temperature range studied.

Table 3. Parameters of Arrhenius model fitted to the data of pure samples and blends.

Sample	A (Pa s)	Ea (kJ mol^{-1})	R^2	χ^2
ASO	0.0673	78.99	0.9711	0.0032
30:70	0.0894	84.47	0.9632	0.0054
50:50	0.0816	82.60	0.9656	0.0046
70:30	0.0929	86.45	0.9635	0.0052
CSF	0.0841	82.03	0.9634	0.0054

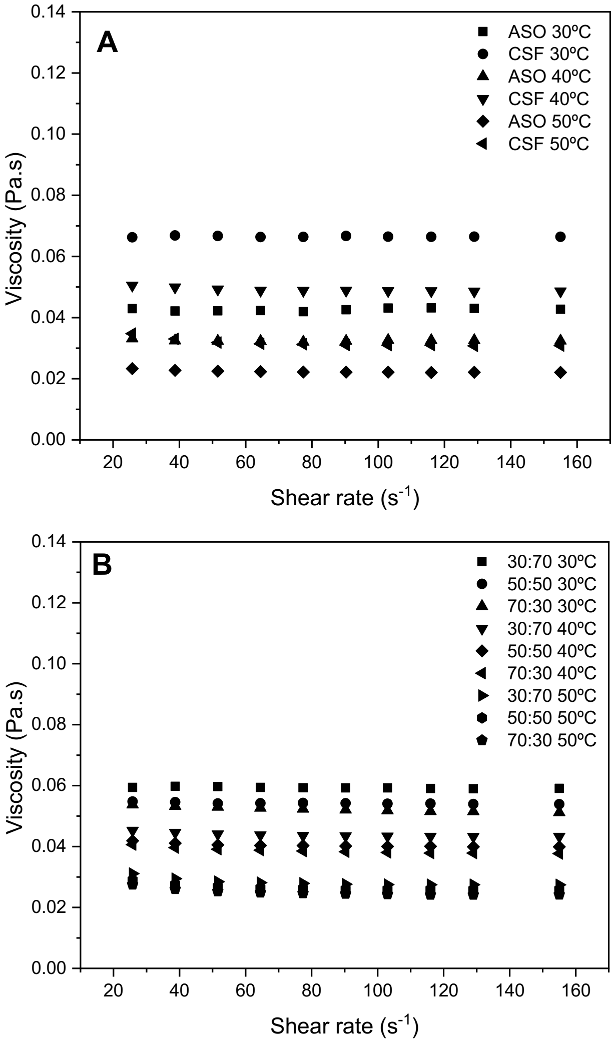

Figure 3. Relationship between viscosity and shear rate of pure samples (A) and blends (B).

Previous studies have attributed the Newtonian fluid behavior, characteristic of oils and fats, to the long-chain molecules that constitute them (Santos *et al.*, 2004). Santos *et al.* (2005) studied the rheological behavior of traditional vegetable oils and their blends and found similar results.

As shown in Figure 3, viscosity remained constant regardless of the shear rates tested, whereas the increase in temperature caused a gradual reduction in viscosity of all samples. The effect of temperature on the viscosity of oils and fats can be attributed to the reduction of intermolecular interactions that occur due to increased thermal molecular motion (Santos *et al.,* 2005).

The viscosity of all samples was inversely proportional to the SV and directly proportional to the IV. This behavior occurs because the viscosity of vegetable oils depends on the fatty acid composition, which increases according to their chain size and decreases with unsaturation (Santos *et al.,* 2004). As expected, ASO presented lower viscosity compared to CSF at all temperatures studied because ASO has a greater content of unsaturated fatty acids (Figure 3 B). Besides, the increase in ASO caused a reduction in the viscosity of the blends. (Figure 3 A).

From an industrial point of view, the decrease in viscosity facilitates the material flow and heat exchange during processing. The lower the viscosity of a fluid, the lower the pressure drop during flow, decreasing power costs with pumping and, consequently, energy costs (Braga *et al.,* 2013).

The Arrhenius equation (Equation 5) adequately represented the effect of temperature on the apparent viscosity of the samples, showing R^2 values higher than 0.96, for both pure samples and mixtures, as can be seen in Figure 4 and Table 4.

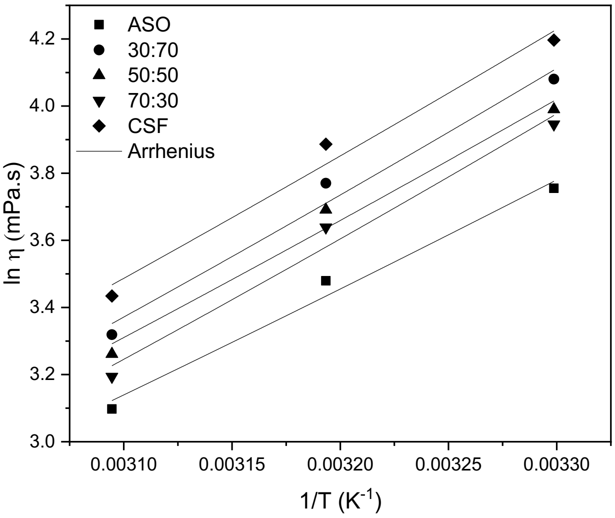

Figure 4. Influence of temperature on the viscosity of pure samples and blends.

The activation energy (E_a) indicates the sensitivity of a material to temperature changes. ASO showed lower E_a (78.99 kJ/mol) than CSF (82.03 kJ mol^{-1}), which indicates that the fat viscosity is more sensitive to temperature changes (Steffe, 1996). The blends exhibited higher E_a results than the pure samples, indicating that the combinations of oil and fat increased their sensitivity to temperature changes.

Regardless of the method of obtaining or using vegetable oils, the knowledge of thermophysical properties, such as viscosity, is of fundamental importance for the achievement of the stages of equipment and process design or even for product specification. In the cosmetics, food and pharmaceutical industries, knowledge of such properties is necessary for the design and development of calculations, equipment and processes involving the production and formulation of new products.

The mixture of fats had a positive effect on viscosity, allowing flexibility in their applications, in addition to providing nutritional properties. While cupuassu seed fat has been used to replace cocoa fat (Medeiros *et al.,* 2006), presenting a higher percentage of saturated fatty acids, annatto fat has more advantages in terms of unsaturated fatty acids, which gives the characteristic viscosity properties to two pure fats. The behavior of the mixture both contributed to a better distribution of the fatty acid profile and allowed an important variation in the viscosity of the fats.

4. CONCLUSIONS

The blends of ASO were demonstrated to have improved nutrition and functionality compared to the individual samples. Greater amounts of ASO increased PUFA content in the blends, whereas greater concentrations of CSF conferred higher MUFA levels. AI and TI results were relatively low, and the blends with greater fat concentrations also showed higher h/H values, suggesting that these samples contain great amounts of fatty acids, which is considered beneficial for human health.

Regarding the spectra of the blends, it was observed that the vibration bands did not undergo a relevant shift in relation to wavelength, indicating that the different combinations did not affect their functional groups.

All samples exhibited Newtonian fluid behavior. The Newtonian model satisfactorily described their rheological behavior in the temperature range studied, according to parameters R^2, χ^2, and RSS.

The blends of ASO and CSF have interesting characteristics, with notable improvements in their nutritional, and functional properties, making them valuable raw materials for the development of new products in the food, pharmaceutical, and cosmetic industries. For example, protective agents and carriers of fat-soluble vitamins, cosmetic emulsions and creams, among other products

ACKNOWLEDGMENTS

The authors would like to thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

CONFLICT OF INTEREST

The authors declare complete absence of conflict of interest between them and the companies *Gran Oils* Industry (São Paulo, Brazil) and *Amazon Oil* Industry (Ananindeua, Pará, Brazil).

REFERENCES

Alparslan M, Hayta M. 2002. Rheological and sensory properties of pekmez (grape molasses)/tahin (sesame paste) blends. *J. Food Eng.* **54**, 89–93. http://doi.org/10.1016/S0260-8774(01)00197-2.

AOCS. 2004. American Oil Chemists' Society. *Official methods and recommended practices of the AOCS*. 4th ed. Champaing.

Attia YA, Al-Harthi MA, Korish MA, Shiboob MM. 2015. Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. *Lipids Health Dis.* **14**, 1–8. https://doi.org/10.1186/s12944-015-0133-z.

Bezerra CV, Rodrigues AMC, Oliveira PD, Silva DA, Silva LHM. 2017. Technological properties of amazonian oils and fats and their applications in the food industry. *Food Chem.* **221**, 1466–1473. https://doi.org/10.1016/j.foodchem.2016.11.004.

Bhattacharjee B, Pal PK, Chattopadhyay A, Bandyopadhyay D. 2020. Oleic acid protects against cadmium induced cardiac and hepatic tissue injury in male Wistar rats: A mechanistic study. *Life Sci.* **244**, 1–18. https://doi.org/10.1016/j.lfs.2020.117324.

Bitencourt APR, Duarte JL, Oliveira AEMFM., Cruz RAS, Carvalho JCT, Gomes ATA, Ferreira IM, Ribeiro-Costa RM, Silva-Júnior JOC, Fernandes CP. 2018. Preparation of aqueous nanodispersions with annatto (*Bixa orellana* L.) extract using an organic solvent-free and low energy method. *Food Chem.* **257**, 196–205. https://doi.org/10.1016/j.foodchem.2018.02.067.

Braga ACC, Rodrigues AMC, Silva LHM, Araújo LA. 2013. Avaliação da influência da temperatura e do tratamento enzimático no comportamento reológico do suco de abacaxi pérola (*Ananas comosus* L. Merr.). *Rev. Bras. Frutic.* **35**, 226–237. https://doi.org/10.1590/S0100-29452013000100026.

Chen J, Liu H. 2020. Nutritional indices for assessing fatty acids: a mini-review. *Int. J. Mol. Sci.* **21**, 5695. https://doi.org/10.3390/ijms21165695.

Costa CK, Silva CB, Lordello ALL, Zanin SMW, Dias JFG, Miguel MD, Miguel OG. 2013. Identificação de δ tocotrienol e de ácidos graxos no óleo fixo de urucum (*Bixa orellana* Linné). *Rev. Bras. Pl. Med.* **15**, 508–512. https://doi.org/10.1590/S1516-05722013000400006.

Dymińska L, Calik M, Albegar AMM, Zając A, Kostyń K, Lorenc J, Hanuza J. 2016. Quantitative determination of the iodine values of unsaturated plant oils using infrared and Raman spectroscopy methods. *Int. J. Food Prop.* **20**, 2003–2015. https://doi.org/10.1080/10942912.2016.1230744.

Fernandes CE, Vasconcelos MAS, Ribeiro MA, Sarubbo LA, Andrade SAC, Filho ABM. 2014. Nutritional and lipid profiles in marine fish species from Brazil. *Food Chem.* **160**, 67–71. https://doi.org/10.1016/j.foodchem.2014.03.055.

García-González DL, Baeten V, Pierna JAF, Tena N. 2013. *Handbook of Olive Oil* (2nd ed). New York: Springer US, (Chapter 10).

Hashempour-Baltork F, Torbati M, Azadmard-Damirchi S, Savage GP. 2016. Vegetable Oil Blending: A Review of Physicochemical, Nutritional and Health Effects. *Trends Food Sci. Tech.* **57**, 52–58. https://doi.org/10.1016/j.tifs.2016.09.007.

Medeiros ML, Ayrosa AMIB, Pitombo RNM, Lannes SCS. 2006. Sorption isotherms of cocoa and cupuassu products. *J. Food Eng.* **73**, 402–406.

Rao MP, Manjunath K, Bhagawati ST, Thippeswamy BS. 2014. Bixin loaded solid lipid nanoparticles for enhanced hepatoprotection – Preparation, characterisation and in vivo evaluation. *Int. J. Pharm.* **473**, 485–492. https://doi.org/10.1016/j.ijpharm.2014.07.027.

Rao PP, Rao GN, Jyothirmayi T, Satyanarayana A, Karuna MSL, Prasad RBN. 2015. Characterisation of seed lipids from *Bixa orellana* and Trachyspermum copticum. *J. Am. Oil Chem. Soc.* **92**, 1483-1490. https://doi.org/10.1007/s11746-015-2717-1.

Rodrigues AMC, Darnet S, Silva LHM. 2010. Fatty acid profiles and tocopherol contents of buriti (Mauritia flexuosa), patawa (Oenocarpus bataua), tucuma (Astrocaryum vulgare), mari (Poraquei baparaensis) nad inaja (Maximiliana maripa) fruits. *J. Brazil. Chem. Soc.* **21**, 2000–2004. http://dx.doi.org/10.1590/S0103-50532010001000028.

Santos JCO, Santos IMG, Souza AG. 2005. Effect of heating and cooling on rheological parameters of edible vegetable oils. *J. Food Eng.* **67**, 40–405. https://doi.org/10.1016/j.jfoodeng.2004.05.007.

Santos JCO, Santos IMG, Conceição MM, Porto SL, Trindade MFS, Souza AG, Prasad S, Fernandes VJJ, Araújo AS. 2004. Thermoanalytical, kinetic and rheological parameters of commercial edible vegetable oils. *J. Therm. Anal. Calorim.* **75**, 419–428. https://doi.org/10.1023/b:jtan.0000027128.62480.db.

Santos-Silva J, Bessa RJB, Santos-Silva F. 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lamb: II Fatty acid composition of meat. *Livest. Prod. Sci.* **77**, 187–194. https://doi.org/10.1016/S0301-6226(02)00059-3.

Serra JL, Rodrigues AMC, Freitas RA, Meirelles AJA, Darnet SH, Silva LHM. 2019. Alternative sources of oils and fats from Amazonian plants: Fatty acids, methyl tocols, total carotenoids and chemical composition. *Food Res. Int.* **116**, 12–19. https://doi.org/10.1016/j.foodres.2018.12.028.

Silva DA, Santos PH, Pena RS. 2017. Colloidal and rheological behavior of aqueous dispersions of buriti tree (Mauritia flexuosa) gum. *Emir J. Food Agric.* **29**, 716–723. https://doi.org/10.9755/ejfa.2017.v29.i9.115.

Silva GF, Gamarra GMC, Oliveira AL, Cabral FA. 2008. Extraction of bixin from annatto seeds using supercritical carbon dioxide. *Braz. J. Chem. Eng.* **25**, 419–426. https://doi.org/10.1590/S0104-66322008000200019

Silva-Júnior AA, Scarpa MV, Pestana KC, Mercuri LP, Matos JR, Oliveira AG. 2008. Thermal analysis of biodegradable microparticles containing ciprofloxacin hydrochloride obtained by spray drying technique. *Thermochim. Acta* **467**, 91–98. https://doi.org/10.1016/j.tca.2007.10.018.

Sivakanthan S, Jayasooriya AP, Madhujith T. 2019. Optimization of the production of structured lipid by enzymatic interesterification from coconut (*Cocos nucifera*) and sesame (Sesamum indicum) oils using Response Surface Methodology. *LWT - Food Sci. Technol.* **101**, 723–730. https://doi.org/10.1016/j.lwt.2018.11.085.

Shen J, Yang T, Tang Y, Guo T, Guo T, Hu T, Luo F, Lin Q. 2021. δ-Tocotrienol induces apoptosis and inhibits proliferation of nasopharyngeal carcinoma cells. *Food Funct.* 12, 6374–6388. https://doi.org/10.1039/D1FO00461A.

Steffe JF. 1996. *Rheological Methods in Food Process Engineering.* 2nd ed. Freeman Press, Michigan.

Stringheta PC, Silva PI, Costa AGV. 2018. Annatto/Urucum - *Bixa orellana*. *Exotic Fruits*, 23–30. https://doi.org/10.1016/b978-0-12-803138-4.00006-x.

Ulbricht TLV, Southgate DAT. 1991. Coronary heart disease: seven dietary factors. *Lancet* **338**, 985–992. https://doi.org/10.1016/0140-6736(91)91846-M.

Wołoszyn J, Haraf G, Okruszek A, Wereńska M, Goluch Z, Teleszko M. 2020. Fatty acid profiles and health lipid indices in the breast muscles of local Polish goose varieties. *Poult. Sci.* **99**, 1216–1224. https://doi.org/10.1016/j.psj.2019.10.02.