

# Reference Material 8404

## Almond Flour for Allergen Detection

### REFERENCE MATERIAL INFORMATION SHEET

**Purpose:** This reference material (RM) is intended for harmonizing measurements of allergenic almond proteins in foods with the following understanding: (1) that the use of almonds from different sources or that have undergone different pretreatments (e.g., defatting, roasting) may have different compositions, and (2) if the material is subjected to preparation or processing (e.g., baking, boiling, frying) the composition and properties of the material may change.

**Description:** A unit of RM 8404 consists of five (5) packets of blanched almond flour. Each packet contains approximately 5 g of material.

**Non-Certified Values:** Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [1]. Non-certified values for proximates are provided on an as-received basis in Table 1. The non-certified values are based on results from an interlaboratory comparison [2]. The non-certified value for protein also contains additional data obtained through a collaborating laboratory. These non-certified values are metrologically traceable to the materials and procedures used in their determination.

Table 1. Non-Certified Values for Proximates in RM 8404

Measurand	Mass Fraction <sup>(a)</sup>	Units
Protein	23.29 ± 0.46	%
Fat	56.0 ± 8.0	%
Solids	96.0 ± 1.6	%
Ash	2.85 ± 0.35	%
Calories	648 ± 35	kcal/100 g

<sup>(a)</sup> These values are expressed as  $x \pm 2u(x)$ , where  $x$  is the value and  $u(x)$  is the standard uncertainty of  $x$ . The standard uncertainty combines between laboratory reproducibility and between unit heterogeneity. While the best estimate of the mass fraction for all measurands lies within the interval  $x \pm 2u(x)$ , this interval may not include the true value. For guidance in propagating this uncertainty, see reference 3.

**Period of Validity:** The non-certified values are valid within the measurement uncertainty specified until **31 December 2026**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

**Maintenance of Non-Certified Values:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the <https://www.nist.gov/srm> website.

**Safety:** RM 8404 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

**Storage:** The original unopened packets of RM 8404 should be stored at  $-20\text{ }^{\circ}\text{C}$ . The value assignment only applies to the initial use of each packet, and the same results are not guaranteed if the remaining material is used at a later date.

**Use:** Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly. Test portion sizes should be appropriate to the measurands and analytical methods selected. The unopened packet should be allowed to warm to room temperature ( $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ) immediately prior to use. The almond powder should not be irradiated, heated, or otherwise treated in any way that might alter the proteins present.

**Homogeneity Assessment:** The homogeneity of RM 8404 was assessed as part of the value assignment for protein as described in Table 1. Additional information about elemental composition was collected during the packaging process [4]. Analysis of variance of 10 replicate measurements across the batch at a 5 % significance showed no statistically significant heterogeneity. Homogeneity of other constituents was not assessed.

**Other Information of Potential Interest:** Additional information from testing and evaluation of RM 8404 for the presence of other allergens is provided in Appendix A. Data in Appendix A may be of interest to the RM user, but insufficient information is available to assess associated uncertainties, therefore no uncertainties are provided. This information cannot be used to establish metrological traceability to the SI or other higher-order reference systems.

## REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Diewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed Jan 2022).
- [2] Barber, C.A.; Burdette, C.Q.; Hayes, H.V.; Luvonga, C.; Phillips, M.M.; Rimmer, C.A.; Wood, L.J.; Yu, L.L.; *Health Assessment Measurements Quality Assurance Program: Exercise 5 Final Report*; NIST Internal Report (NIST IR) 8343; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/ir/2021/NIST.IR.8343.pdf> (accessed Jan 2022).
- [3] Possolo, A.; *Evaluating, Expressing, and Propagating Measurement Uncertainty for NIST Reference Materials*; NIST SP 260-202; U.S. Government Printing Office: Washington, DC (2020); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-202.pdf> (accessed Jan 2022).
- [4] Phillips, M.M.; Bunk, D.M.; Green, A.B.; Yen, J.H.; *Value Assignment of Reference Material 8404 Almond Flour for Allergen Detection*; NIST SP 260-218; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-218.pdf> (accessed Jan 2022).

### If you use this RM in published work, please reference:

Phillips MM, Bunk DM, Green AB, Yen JH (2021) Value Assignment of Reference Material 8404 Almond Flour for Allergen Detection. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-218. <https://doi.org/10.6028/NIST.SP.260-218>

*Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.*

*Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>.*

\* \* \* \* \* End of Reference Material Information Sheet \* \* \* \* \*

# APPENDIX A

All testing results and customer feedback regarding the potential contamination of RM 8404 with other trace allergens is summarized in the table below. Observed presence of macadamia nut is most likely explained as cross-reactivity of the 3M and Romer assays to the high levels of almond protein and, therefore, not of concern with respect to the protein content of RM 8404.

Allergen	Presence mg/kg (ppm)	Based on
β-Lactoglobulin	<0.031	Eurofins Analytik GmbH ELISA
Brazil Nut	<1 <1 <4	3M ELISA BioFront MonoTrace ELISA Eurofins Analytik GmbH ELISA
Casein	<0.25 <0.4	Eurofins Analytik GmbH ELISA R-Biopharm SureFood PCR
Cashew	<0.2 <0.9 <1 <2 ND	Romer US-AgraQuant ELISA 3M ELISA BioFront MonoTrace ELISA Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR
Celery	ND	Eurofins Analytik GmbH PCR
Coconut	<2 <2	3M ELISA Eurofins Analytik GmbH ELISA
Egg	<0.31	Eurofins Analytik GmbH ELISA
Fish	ND	Eurofins Analytik GmbH PCR
Hazelnut	<0.16 <0.3 <0.4 <1 <1 <2.5 <2.5 ND	Eurofins Analytik GmbH ELISA Romer US-AgraQuant ELISA R-Biopharm SureFood PCR BioFront MonoTrace ELISA 3M ELISA R-Biopharm ELISA Neogen Veratox ELISA Eurofins Analytik GmbH PCR
Lupin	<2 ND	Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR
Macadamia	<0.3 <b>0.5</b> <b>35</b> <1 <2	3M ELISA <b>3M ELISA</b> <b>Romer US-AgraQuant ELISA</b> Eurofins Analytik GmbH ELISA BioFront MonoTrace ELISA
Mustard	<2 ND	Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR

Allergen	Presence mg/kg (ppm)	Based on
Oat	ND	Eurofins Analytik GmbH PCR
Peanut	<0.1 <0.2 <0.4 <1 <2.5 ND	Romer US-AgraQuant ELISA Eurofins Analytik GmbH ELISA R-Biopharm SureFood PCR BioFront MonoTrace ELISA Neogen Veratox ELISA Eurofins Analytik GmbH PCR
Pecan	<0.4 <0.66 <1 <1 <2	R-Biopharm SureFood PCR 3M ELISA Romer US ELISA BioFront MonoTrace ELISA Eurofins Analytik GmbH ELISA
Pine Nut	<0.7 <1	Romer US ELISA BioFront MonoTrace ELISA
Pistachio	<0.1 <0.4 <1 <1 ND	Romer US-AgraQuant ELISA R-Biopharm SureFood PCR BioFront MonoTrace ELISA 3M ELISA Eurofins Analytik GmbH PCR
Sesame	<2 ND	Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR
Soy	<0.31 ND	Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR
Walnut	<0.3 <0.4 <1 <2 <2 ND	Romer US-AgraQuant ELISA R-Biopharm SureFood PCR BioFront MonoTrace ELISA 3M ELISA Eurofins Analytik GmbH ELISA Eurofins Analytik GmbH PCR
Wheat	<2 <3 ND	Romer US-AgraQuant ELISA Romer US-RIDASCREEN Eurofins Analytik GmbH PCR

\*\*\*\*\* End of Appendix A \*\*\*\*\*