OCLC 1482120 **No holdings in XXX - 877 other holdings**

Continuing Resources

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510 2 Chemical abstracts $x 0009-2258
555 Vols. 1-75, 1942-1958. 1 v. (Includes index to the journal under its earlier title).

580 The journal: Nitric oxide, is also called: Archives of biochemistry and biophysics. Part B.
580 Absorbed: Molecular cell biology research communications, Nov. 2001.
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650 6 Biophysique $v Périodiques.
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Attachment 21d

LIBRARY OF CONGRESS AUTHORITIES

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Attachment 22a

MARC Bibliographic Record

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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U.S. Patent No. 11,224,241

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Case No.: IPR2023-00207
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500          $aRefereed/Peer-reviewed
525          $aSome issues accompanied by supplementary materials or supporting information.
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Continuing Resources

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530 Also issued on microfiche, microfilm, and online.
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Attachment 23a

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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500        $aPlace of publication varies.
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Case No.: IPR2023-00207
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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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525 Some issues <1978-Jan. 1995 accompanied by supplementary material on microfiche; Feb. 1995-<Feb. 1998, by supporting information on microfiche; current (1995-) supporting information also available online.
530 Also issued on microfiche, microfilm, and online.
550 Volumes for 1953-<54 published by the American Chemical Society; <Jan./Feb. 1976- by the Books and Journals Division, American Chemical Society.
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Attachment 24a

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530          $aAlso available online via the World Wide Web by subscription.
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362 0_ $aVol. 14, issue 3 (Mar. 1996)-

500 $aRefereed/Peer-reviewed

500 $a"Some content from issues published prior to the November 1998 re-launch of Nature Biotechnology Online ma not be available in full-text or PDF format."

500 $aTitle from title screen (viewed Aug. 5, 1999).

525 $aSupplements accompany some volumes; issues for <Vol. 21 (2003)> have occasional supplement: Bioentrepreneur. Beginning in 2003? Bioentrepreneur published as a separate "portal."

538 $aMode of access: World Wide Web.

580 $aComplements the journal: Nature.

588 1_ $aVol. 38, no. 1 (Jan. 2020) (Nature website, viewed Jan. 27, 2020).

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Attachment 24e

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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500 Title from cover.
510 2 Chemical abstracts $x 0009-2258
525 Supplements accompany some volumes; issues for <Vol. 21 (2003) have occasional supplement: Bioentrepreneur.
530 Also issued in microform by University Microfilms, Inc.
530 Also issued online via the World Wide Web by subscription.
580 Complements the journal Nature with articles for an audience specialized in biotechnology.
588 1 Vol. 21, no. 11 (Nov. 2003).
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Attachment 25a

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

ScienceDirect

Food Chemistry: X
Volume 3, 30 September 2019, 100038

Streamlined approach for careful and exhaustive aroma characterization of aged distilled liquors

Wenqi Zhu, Keith R. Cadwallader

Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 1302 West Pennsylvania Avenue, Urbana, IL 61801, USA

Received 1 February 2019, Revised 4 June 2019, Accepted 8 June 2019, Available online 11 June 2019, Version of Record 17 June 2019.

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Highlights

- A careful and exhaustive streamlined method for aroma analysis of distilled liquors was developed.
- Perceived aromas did not differ between SAFE distillates and original (neat) liquors.
- Accurate quantitation was achieved for aroma compounds of high and intermediate volatility.
- Less accurate results were obtained by the streamlined method for semi-volatile components.

Abstract

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Solvent-assisted flavor evaporation (SAFE) is considered to be the best overall method to produce a "clean" aroma extract to avoid the loss of labile aroma compounds or the formation of thermally generated artifacts during gas chromatographic (GC) analysis. However, SAFE is both time consuming and labor intensive, especially when applied repeatedly for quantitation by stable isotope dilution analysis (SIDA), which requires the addition of isotopes within specific mass ratio ranges relative to target analytes. The streamlined approach described herein allows for accurate quantitation of odor-active components in liquor products with a single SAFE operation. The quantitative results achieved by this method are nearly identical for most odor-active components, except for specific semi-volatile constituents not recovered well by SAFE (e.g., vanillin and syringaldehyde in oak-aged liquors). The streamlined approach provides a simple and convenient way to expedite the careful and exhaustive study of the flavor chemistry of aged liquors.

Previous Next

Keywords

Solvent-assisted flavor evaporation (SAFE); Stable isotope dilution analysis (SIDA); Aroma extract dilution analysis (AEDA); Gas chromatography–mass spectrometry-olfactometry (GC–MS-O)

1. Introduction

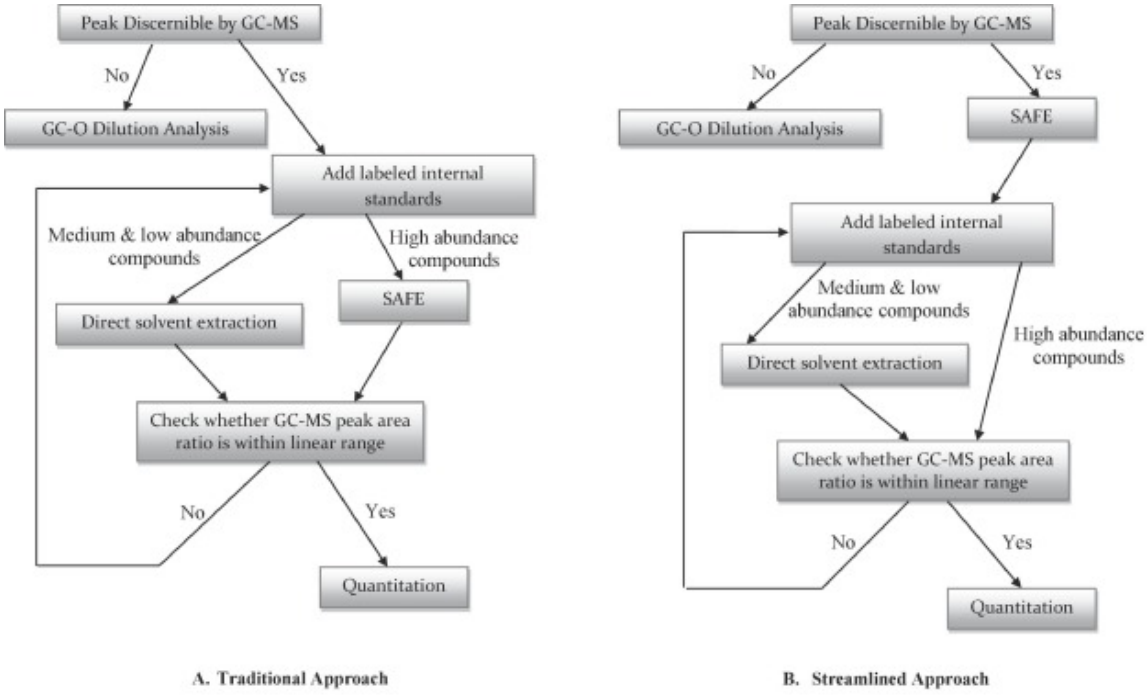
Study of the flavor chemistry of distilled spirits and liquors by GC has a long history of several decades. The accurate identification and quantitation of the aroma components of liquor products is of significant importance in determining product quality and authenticity and for optimization of brewing and blending technology (MacNamara & Hoffmann, 1998). Among the various techniques applied for volatile compound isolation and quantitation, solvent-assisted flavor evaporation (SAFE) combined with gas chromatography-olfactometry (GC-O) and stable isotope dilution analysis (SIDA) is considered the state-of-the-art approach, which has been widely applied in recent studies on the flavor chemistry of liquor products (Franitza et al., 2016, Lahne., 2010, Poisson and Schieberle, 2008). SAFE generates authentic "clean" extracts suitable for careful on-column GC analysis, a procedure which minimizes losses of labile aroma compounds or generation of thermally generated artifacts (Engel, Bahr, & Schieberle, 1999). In the case of aged liquors the use of SAFE is essential if on-column GC analysis is to be used since the solvent extract may contain nonvolatile compounds, such as oak-derived nonvolatile compounds in the case of aged whiskeys. Even some liquors that are aged in pottery, e.g. Chinese liquors (baijius), may contain some nonvolatile compounds. A case in point is Moutai, a traditional Chinese baijiu, which is known to contain lichenysin, a nonvolatile cyclooctapeptide produced by *Bacillus licheniformis* during fermentation (Zhang et al., 2014, Zhang et al., 2014).

Meanwhile SIDA, which makes use of a deuterium or carbon-13 labeled analogue of a target analyte as the internal standard for quantitation, corrects for extraction bias – which occurs to some extent with all volatile isolation techniques including SAFE – thus providing highly accurate results. Due to the great similarity of the isotopes and their corresponding analytes with respect to their physical and chemical

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properties, their mass ratios remain essentially unchanged before and after extraction and during subsequent sample workup protocols. For the above reasons, SAFE and SIDA serve as the gold standards for volatile isolation and quantitation, respectively.

A typical procedure for the flavor analysis of a food product by using SAFE combined with SIDA is as follows (Fig. 1A): isotopes are added to the known amount of product and equilibrated followed by solvent extraction; The solvent extract is then subjected to SAFE and the peak area ratios of the isotopes and target analytes are determined by GC–MS to provide guidance for the adjustment of isotope addition if needed. To establish the ratio of each isotope to its corresponding target analyte within the linear range of the standard curve, this "addition-extraction-adjustment" procedure is repeated several times before finally the correct level of isotope addition is determined. The whole procedure is tedious and time consuming, and expensive if the isotopes and target product are costly, and possibly impractical if the target product is scarce or otherwise in limited supply. Thus, optimizing the efficiency of this quantitative analysis method is essential for reducing the time and cost, as well as to maintain the accuracy of this state-of-the-art methodology.



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Fig. 1. Flowcharts of traditional (A) and streamlined (B) approaches used for stable isotope dilution analysis (SIDA).

In the SAFE procedure, odor-active components are distilled under high vacuum, thus, the extraction bias during SAFE is based on volatility rather than polarity, like in the case of solvent extraction. If volatile losses during SAFE are negligible the direct SAFE distillate could be subjected to SIDA to allow the adjustment of isotope addition performed in a small scale to reduce the number of SAFE operations and the time and costs associated with isotopes and potentially limited sample quantities.

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In the present study, isotopes were added after SAFE extraction was completed instead of before any extraction procedures as in the standard protocol (Lahne., 2010, Poisson and Schieberle, 2008). To assess the feasibility of this streamlined procedure (Fig. 1B) and to identify potential pitfalls and limitations, a series of experiments were conducted. The standard protocol has limitations regarding quantitation of aroma components of scarce or expensive products. Aged distilled liquor is good a case in point and was selected as the target product in this study. To have a better assessment of the potential of the streamlined procedure, three clear liquors (Chinese baijius) aged in porcelain and three brown liquors (whiskey, tequila and rum) aged in oak barrels, were selected to assess and validate the procedure in the present study. Liquor samples were compared with their counterpart SAFE distillation isolates by sensory and instrumental methods to assess the advantages and limitations of the proposed streamlined approach.

2. Materials and methods

2.1. Materials

All the liquors used in this study were commercial products. The selected clear distilled liquors included the top soy sauce aroma liquor Moutai, which is also the national liquor of China (MT, Kweichow Moutai Co. Ltd. Guizhou, China.), the top sesame flavor liquor Yi Pin Jing Zhi (YPJZ, Jingzhi Liquor Co., Ltd., Shandong, China.) and a mid-range strong aroma liquor Gu Jing Gong Jiu (GJGJ, Gujinggong Liquor Co., Ltd. Bozhou, China.). These liquors represent top and medium level clear liquor products which are aged in pottery. For the oak aged (or brown) liquors, Evan Williams Kentucky Bourbon Whiskey (EWW, Old Evan Williams Distillery, Kentucky, U.S.A.), Don Julio Tequila (DJT, Tequila Don Julio, S.A. DE C.V. Jalisco, Mexico.) and Appleton Estate Jamaica Rum (Aged 12 years) (AER, J.Wray & Nephew LTD. Jamaica.) were selected to represent high and mid-range liquor products which are aged in oak barrels. Mention of brand name is not for advertisement or endorsement purposes and does not imply any research contract or sponsorship.

2.2. Chemicals

All authentic reference standards and reagent grade chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. 2-Methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

The following isotopically labeled compounds were purchased from the suppliers listed in parentheses: [$^{2}H_5$]-propanoic acid (Cambridge Isotope Laboratories, Inc., Andover, MA, USA); [*1,2*-$^{13}C_2$]-phenylacetic acid and [1,2-$^{13}C_2$]-butanoic acid (Isotec, Miamisburg, OH, USA); [$^{2}H_9$]-pentanoic acid; [*2,2*-$^{2}H_2$]-3-methylbutanal (CDN Pointe-Claire, Quebec, Canada). [$^{2}H_3$]-acetic acid (CDN, Pointe-Claire, Quebec, Canada).

The following labeled and unlabeled compounds were synthesized according to procedures reported in the literature (in parentheses): 3-methyl-2,4-nonadione (Guth & Grosch, 1989); [*1,2*-$^{13}C_2$]-2-phenylethanol (Schuh & Schieberle, 2006); *bis*(2-methyl-3-furyl) disulfide (Hofmann, Schieberle, & Grosch, 1996); [$^{13}C_2$]-sotolon (Blank, Lin, Fumeaux, Welti, & Fay, 1996); 4-hydroxy-3-[$^{2}H_3$]-methoxybezaldehyde ([$^{2}H_3$]-vanillin) (Schneider & Roland, 1992); [$^{2}H_4$]-β-damascenone (Kotseridis, Baumes, & Skouroumounis, 1998); 2-methyl-[2,3-$^{2}H_2$]-propan-1-ol, [*2,3*-$^{2}H_2$]-methylpropanal and [*3,4*-$^{2}H_2$-2-methylbutanal (Wu & Cadwallader, 2019).

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2.2.1. Synthesis of [3,3,4,4]-$^2H_4$-hexanoic acid

3-Hexyn-1-ol was deuterated to [3,3,4,4-$^2H_4$]-hexan-1-ol using a previously described method (Hausch, Lorjaroenphon, & Cadwallader, 2015). The deuterated alcohol was then oxidized to the corresponding acid using potassium permanganate as described previously (Guth & Grosch, 1994) for the synthesis of [3,4-$^2H_2$]-3-methylbutyric acid. MS-EI, *m/z (%)* of [3,3,4,4-$^2H_4$]-hexanoic acid (purity 98.3%): MS-EI, *m/z(%)*: 61 (100), 75 (39), 60 (29) , 44 (25), 43 (22), 45 (20), 62 (14), 74 (11).

2.2.2. Synthesis of [2,3-$^2H_2$]-2-methylpropanoic acid

2-Methyl-*[2,3-$^2H_2$]*-propan-1-ol was oxidized to the corresponding acid using potassium permanganate as described previously (Guth & Grosch, 1994) for the synthesis of [3,4-$^2H_2$]-3-methylbutanoic acid. MS-EI, *m/z(%)* of [2,3-$^2H_4$]-2-methylpropanoic acid (purity 99.8%): 61 (100), 75 (39), 60 (29) , 44 (25), 43 (22), 45 (20), 62 (14), 74 (11).

2.2.3. Synthesis of 4-hydroxy-3[$^2H_3$],5-dimethoxy benzaldehyde ($^2H_3$-syringaldehyde)

In a 50-mL screw-capped test tube (PTFE-cap) equipped with a stir bar, 3,4-dihydroxy-5-methoxybenzaldehyde (0.501 g; 3 mmol) was dissolved in aqueous 40% (w/v) KOH (5 mL). Then, under a gentle steam of nitrogen and over the course of 30 min, 0.35 mL (0.42 g, 3.2 mmol) of [$^2H_6$]-dimethylsulfate was added (5 to 6 drops every 5 min) to the reaction tube, after which the reaction mixture became yellow and cloudy. The vial was then capped and stirred for 2 h. The reaction was checked for completion by removing 5–6 drops of the reaction mixture, adding it to a vial containing 1 mL aqueous 1 N HCl and 0.5 mL ethyl acetate, and then analyzing the ethyl acetate layer by GC–MS. The reaction was continued by adding 0.08 mL (0.096 g, 0.73 mmol) of [$^2H_6$]-dimethylsulfate and allowing the reaction to stir overnight until nearly all starting material had been consumed. The reaction was stopped by acidifying the mixture to ~pH 1 and then it was extracted with ethyl acetate (1×10 mL, 4×5 mL). The ethyl acetate layer was washed with saturated NaCl and then dried over anhydrous Na_2SO_4. The solution was concentrated to ~10 mL using a vigreux column and the remaining solvent was then removed under a stream of nitrogen. The final product was weighed for a final yield of 0.5734 g (purity 98.1%). MS-EI, *m/z (%)* of [$^2H_3$]-syringaldehyde: 185 (100), 184 (66), 96 (20), 39 (15), 51 (14), 114 (14), 67 (13), 53 (12), 79 (12), 111 (11), 186 (11), 68 (10), 95 (10), 50 (10).

2.2.4. Synthesis of [$^2H_5$]-ethyl esters – general procedure

The following reagents were added to a 20-mL screw top test tube: 10 mmol of organic acid; 200 μL of [$^2H_6$]-ethanol (158 mg; 3 mmol) and 2 drops concentrated H_2SO_4. The test tube was sealed with PTFE-lined screw cap and placed in a large bottle or beaker to protect against breakage and then incubated in a 100 °C oven for 2 h. After cooling to room temperature, the mixture was diluted with pentane (10 mL) and then 5 mL of aqueous saturated Na_2CO_3 solution was added. The aqueous layer was removed the pentane layer was washed again with Na_2CO_3 (5 mL). (This step was necessary in order to remove any unreacted acid.). The pentane layer was washed with aqueous saturated NaCl (2×5 mL) and then dried over anhydrous Na_2SO_4. The solvent (pentane) was evaporated off to yield the ester, generally in high purity (>99%). If needed, the ester was further purified by flash chromatography on silica gel using 100% pentane as elution solvent.

[$^2H_5$]-ethyl phenylacetate (purity 98.7%)

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MS-EI, *m/z (%)*:91 (100), 169 (31, M+), 92 (14), 65 (11), 170 (5), 93 (5), 89 (5), 39 (4)

[$^2H_5$]-ethyl 3-phenylpropanoate (purity 99.7%)

MS-EI, *m/z (%)*: 104 (100), 107 (51), 105 (50), 183 (45, M+), 91 (45), 77 (19), 79 (18), 92 (15), 133 (13), 103 (13), 51 (10)

[$^2H_5$]-ethyl butanoate (purity 99.7%)

MS-EI, *m/z (%)*: 43 (100), 71 (88), 93 (62), 41 (35), 61 (27), 42 (24), 74 (23), 39 (15), 106 (10), 44 (10).

[$^2H_5$]-ethyl 3-methylbutanoate (purity 99.3%)

MS-EI, *m/z (%)*: 93 (100), 57 (70), 85 (59), 61 (50), 41 (48), 43 (28), 39 (23), 42 (23), 63 (16), 59 (14), 120 (13), 74 (12), 88 (12), 46 (12), 56 (11), 44 (10), 73 (10).

[$^2H_5$]-ethyl pentanoate (purity 98.8%)

MS-EI, *m/z (%)*: 93 (100), 85 (86), 57 (77), 61 (58), 74 (48), 41 (44), 106 (37), 55 (25), 56 (21), 63 (21), 39 (18), 73 (15), 42 (15), 64 (11), 49 (10).

[$^2H_5$]-ethyl hexanoate (purity 98.0%)

MS-EI, *m/z (%)*: 93 (100), 43 (64), 99 (46), 61 (41), 74 (34), 41 (33), 106 (29), 42 (26), 71 (22), 55 (22), 39 (15), 63 (14), 73 (10), 44 (10), 120 (10).

[$^2H_5$]-ethyl heptanoate (purity 99.8%)

MS-EI, *m/z (%)*: 93 (100), 43 (52), 61 (37), 41 (36), 74 (33), 113 (32), 106 (31), 55 (26), 42 (15), 63 (15), 120 (15), 56 (13), 39 (13), 57 (10).

[$^2H_5$]-ethyl octanoate (purity 99.8%)

MS-EI, *m/z (%)*: 93 (100), 106 (34), 57 (34), 74 (33), 41 (32), 61 (29), 55 (28), 127 (23), 43 (22), 42 (17), 63 (12), 56 (10).

[$^2H_5$]-ethyl decanoate (purity 97.4%)

MS-EI, *m/z (%)*:93 (100), 106 (44), 74 (31), 41 (28), 43 (25), 55 (24), 61 (20), 162 (14), 155 (11), 57 (11), 63 (11), 42 (10), 94 (10).

[$^2H_5$]-ethyl dodecanoate (purity 99.9%)

MS-EI, *m/z (%)*: 93 (100), 106 (52), 74 (27), 41 (27), 43 (25), 55 (25), 61 (14), 57 (14), 94 (13), 162 (10).

[$^2H_5$]-ethyl hexadecanoate (purity 92.8%)

MS-EI, *m/z (%)*: 93 (100), 106 (61), 43 (32), 55 (27), 41 (25), 74 (21), 57 (19), 94 (18), 162 (13), 69 (12), 61 (10).

3. Methods

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3.1. Direct distillation of liquor (SAFE-DIST)

Liquor sample (100 mL) was fed directly into the SAFE apparatus as previously described (Rotsatchakul, Chaiseri, & Cadwallader, 2007). The system was maintained under high vacuum (2–5 × 10^{-5} Torr) and at 40 °C throughout the 2 h distillation process. The distillate was stored in a glass bottle equipped with a PTFE-lined cap.

3.2. Sensory methodology

Sensory testing was approved (protocol number 17507) by the Institutional Review Board (IRB) of the University of Illinois at Urbana-Champaign. Panelists (24), consisting of 16 females and 8 males, ranging in age from 21 to 55, were selected to participate in triangle difference testing to determine whether the overall aroma characteristics of the SAFE-DIST isolates differed from the original (neat) liquor products. SAFE isolates and liquor products (20 mL each) were transferred into 125-mL Teflon sniff bottles (Nalgene PTFE wash bottle without a siphon tube; Nalge Nunc International, Rochester, NY, USA) which were wrapped with aluminum foil to prevent visual bias and to protect the liquors from light exposure. For triangle difference testing (Meilgaard & Civille, 2007), samples were presented to panelists in two orders: one set consisted of two SAFE-DIST isolates and one original (neat) liquor product and the other consisted of one SAFE-DIST isolate and two original (neat) liquor products. Panelists were asked to assess the aroma properties of each sample and choose the odd sample.

3.3. GC-FID analysis

Original liquors and their counterpart SAFE-DIST isolates were analyzed using a 6890N GC-equipped with a flame-ionization detector (FID) (Agilent Technologies, Inc., Santa Clara, CA, USA). Separations were performed using an Rtx-Wax column (15 m × 0.53 mm i.d. × 1 μm film thickness; Restek Corp., Bellefonte, PA, USA). Analyses were conducted in triplicate to assure accurate and precise measurements. The samples were injected in hot split mode (1:10) with an inlet temperature of 250 °C. The carrier gas was helium at a flow rate of 2 mL/min. The oven temperature was programmed from 35 °C to 225 °C at a ramp rate of 10 °C/min with initial and final hold times of 5 and 20 min, respectively. Peak areas for selected compounds found in moderate to high abundance were compared across the original liquors and their counterpart SAFE-DIST isolates.

3.4. Gas chromatography–mass spectrometry-olfactometry (GC–MS-O)

One clear liquor (MT) and one brown liquor (EWW) were analyzed to compare the aroma profiles before and after SAFE distillation. Odor-active components in liquor products and their respective SAFE isolates were extracted by direct solvent extraction (DSE). SAFE distillate or neat liquor (7.5 mL) was pipetted into a 50-mL glass centrifuge tube containing 40 mL of odor-free deionized-distilled water and 0.5 mL of DCM. The mixture was shaken vigorously for 5 min and centrifuged at 4500 rpm for 10 min. The extraction procedure was repeated two more times. The pooled solvent extract was frozen overnight to remove excess water, then the extract was transferred into a 2-mL vial and condensed to 1 mL using a gentle stream of ultra-high purity nitrogen gas.

GC–MS-O was performed using a 6890N GC/5973N mass selective detector (MSD) system (Agilent Technologies, Inc.). Analyses were performed on both polar (Stabilwax, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.) and nonpolar (Rxi-5ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.)

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columns. Aroma extracts (2 μL) were injected using a CIS-4 (Gerstel, Germany) programmable temperature vaporizer (PTV) inlet in the cold-splitless mode (-50 °C initial temperature (0.1 min hold), ramped at 12 °C/s to 250 °C and held for 20 min). The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 40 °C to 250 °C at a ramp rate of 3 °C/min with initial and final hold times of 5 and 30 min, respectively. Temperatures of MSD transfer line and olfactory port were set at 250 °C. Mass scan range was set as 33–300 amu with scan rate of 5.27 scans/s and electron energy was 70 eV. GC–MS data were analyzed by ChemStation Enhanced Data Analysis Software (Agilent Technologies, Inc.). For tentative compound identifications, mass spectra of the analytes were compared against those in 2008 National Institute of Standards and Technology (NIST) Mass Spectral Library.

3.5. On-column gas chromatography-olfactometry (GC-O)

GC-O was performed using a 6890 GC (Agilent Technologies Inc.) equipped with a flame ionization detector (FID), a sniff port (DATU Technology Transfer, Geneva, NY, USA) and a cool on-column injector (+3 °C, oven tracking). A selected SAFE-DIST (2 μL) was injected directly into either a polar (RTX-Wax, 15 m × 0.32 mm i.d., 0.5 μm film thickness; Restek Corp.) or nonpolar (RTX-5, 15 m × 0.32 mm i.d., 0.5 μm film thickness; Restek Corp.) column. The carrier gas was helium at a constant flow rate of 2 mL/min. The oven temperature was programmed from 40 to 250 °C at a ramp rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. Column effluent was split equally between the sniff port and FID by using 0.15 mm i.d. deactivated capillary columns of equal length (1 m). FID and sniff port temperatures were maintained at 250 °C.

3.6. Compound identification

Retention indices (RI) were calculated based on comparing the retention times of analytes to those determined for a homologous series of n-alkanes (from C_7 to C_{28}) analyzed under the same analytical conditions (van Den Dool & Kratz, 1963). Odorants were identified by comparing their retention indices (RI) on both polar and nonpolar GC columns, mass spectra and odor properties to those of authentic standards. A compound was considered positively identified if all three of the above criteria matched those of a reference standard. However, in some cases, an odorant was considered tentatively identified when one or more of the above criteria could not be met, e.g. when no mass spectrum was available due to the compound being present at a trace level and below that of the detection limit of the MSD, or whenever an authentic standard was not available to confirm an RI, mass spectrum or odor properties. In the latter case, the compound was considered tentatively identified when its RI, mass spectrum and odor properties were in agreement with literature values or mass spectral library database entry.

3.7. Aroma extract dilution analysis (AEDA)

Relative potencies of odor-active compounds were determined by AEDA. DSE extracts were diluted stepwise at a ratio of 1:3 (v/v) in dichloromethane (DCM) and each dilution was analyzed by GC–MS-O as previously described. Flavor dilution (FD) factors of each odorant were determined as the highest dilution at which the odorant was last detected by GCO (Grosch, 2001). The FD factors are shown as $\log_3$ FD-factors for better comparison between each liquor product and its respective SAFE isolate.

3.8. Quantitation by stable isotope dilution analysis (SIDA)

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SIDA was applied to accurately evaluate the recovery of volatile or semi-volatile components in SAFE-DIST. For this purpose, the concentrations of selected volatile components in MT and EWW and their respective SAFE-DIST isolates were determined by SIDA and compared.

Deuterated or carbon-13 labeled isotopes of selected analytes were dissolved in ethanol or DCM and spiked into 1 mL aliquots of neat liquor products or their SAFE-DIST isolates. Sample analysis was performed in the same manner as mentioned previously by using a Stabilwax column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.) and injections were made using the cold-split mode (split ratio 10:1; –50 °C initial temperature (0.1 min hold), ramped at 12 °C/s to 250 °C and held for 20 min). Samples were analyzed under simultaneous full scan (35–300 amu) and selected ion monitoring (SIM) modes. Selected ions used for determination of peak areas of labeled and unlabeled components are listed in Table 1S.

For quantitation of the Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal) in liquor samples, headspace solid-phase microextraction (HS-SPME) was applied. MT (50 μL) or EWW (500 μL) were transferred to a 20-mL SPME vial containing 0.5 g of sodium chloride and 2 mL or 1.5 mL, respectively, of distilled odorless water. After spiking with a proper amount of isotope solution, samples were analyzed by HS-SPME-GC–MS using a 6890N GC/5973N MSD (Agilent Technologies, Inc.) equipped with an MPS2 autosampler (Gerstel) and CS4 injection port (Gerstel). A three phase SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA, USA) was used for volatile extraction. Vials were equilibrated at 60 °C for 20 min followed by a 10 min SPME headspace extraction. Volatiles were desorbed into the GC by hot splitless injection (260 °C; 4 min split valve-delay time). Separations were performed using a RTX-5ms column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.). Helium was used as the carrier gas at 1 mL/min. Oven temperature was programmed as follows: initial temperature, 30° C (5 min hold), ramp rate 6° C/min, final temperature, 225° C (30 min hold).

3.9. Quantitation of sotolon

A special case can be made for sotolon which could be generated in the hot inlet of the GC. Due to its low threshold of 10 ppb in wine and its relatively low concentration in liquor products (Gabrielli et al., 2015), the determination of concentration of sotolon generally requires extraction and compound class fractionation. To determine whether it is an artifact and also to determined its relative recovery by SAFE, sotolon was determined by SIDA using 3 different methods.

1) Isotope solution was added to 10 mL SAFE-DIST followed by direct solvent extraction using DCM (3 × 2 mL).

2) Isotope solution was added to 10 mL liquor product followed by direct solvent extraction using DCM (3 × 2 mL).

3) Isotope solution was added to 10 mL liquor product followed by SAFE distillation (SAFE was operated as mentioned previously) and the distillate underwent direct solvent extraction using DCM (3 × 2 mL).

In all of the above procedures, the DCM extract was washed (3 × 3 mL) with 5% (w/v) aqueous Na_2CO_3. Aqueous phase was then washed with DCM (3 × 5 mL). Then the aqueous phase was adjusted to pH 2 (1 N aqueous H_2SO_4) and then extracted with DCM (3 × 2 mL). Solvent phase was dried over anhydrous Na_2SO_4 (1 g) and condensed to 0.1 mL using a gentle stream of ultra-high purity nitrogen gas. Each method was

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conducted in triplicate and the extracts analyzed using the methods and instrumentation mentioned previously.

3.10. Calibration

The response factor (R*f*) for each isotope against its unlabeled target analyte was determined by a five-point standard curve with a range of mass ratios from 1:5 to 5:1 (unlabeled:labeled). Each point was analyzed in triplicate and the mass ratio of each point was plotted against its mean peak area ratio of selected ions of labeled and unlabeled target analytes.

The R*f* of each labeled analyte was calculated by using the following equation:

$$\frac{\text{mass (analyte)}}{mass\ (labeled\ analyte)} = \frac{area\ (analyte)}{area\ (labeled\ analyte)}\ (Rf)$$

The concentration of each target analyte was calculated by using the following equation:

$$Concentration\ \ (analyte) = \frac{Rf \times Peak\ area\ ratio \times mass\ (labeled\ analyte)}{Sample\ \ volume}$$

3.11. Thermal stability of selected disulfides

Since the aroma extracts were analyzed by split/splitless inlet injection, the volatile components were thus heated and condensed in the inlet which may cause the loss and/or formation of thermally generated artifacts, especially some sulfur compounds. Two sulfur compounds identified in MT, *bis*(2-methyl-3-furyl) (MFT-MFT) and 2-methyl-3-(methyldithio) furan (MFT-MT), were selected and analyzed using various methods to confirm the identification results. For this purpose, potential artifacts caused by different injection techniques were compared by using *n*-alkanes as internal standards which have boiling points close to those of the target analytes. Boiling point of MT-MFT and MFT-MFT are 210 °C and 280 °C, respectively, while the internal standards dodecane (IS for MT-MFT) and hexadecane (IS for MFT-MFT) have boiling points of 215–217 °C and 287 °C, respectively. The disulfides and alkanes were diluted in pentane, at concentrations of 10 μg/mL for MFT-MT, dodecane and MFT-MFT and 1 μg/mL for hexadecane. Solutions were analyzed by GC–MS as described previously using a SAC-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Inc.) and 3 different injection modes using a CIS4 PTV inlet (Gerstel) as earlier described, and by hot-splitless and cool on-column using the same inlet. For hot splitless injection the inlet was set to either 200 °C, 250 °C or 300 °C with split valve delay set to 0.5 min. Inlet temperature for on-column injection was 40 °C. The solution was analyzed in triplicate for each injection condition and the peak areas of each disulfide were compared with the peak areas of its corresponding alkane internal standard. Percent degradation was calculated using the following equation:

$$Degradation\ (\%) = 1 - \frac{Peak\ \ area\ ratio\ \left(\frac{Disulfide}{Internal\ \ standard}\right)}{Peak\ \ area\ ratio\left(\frac{Disulfide}{Interal\ \ standard}\right) by\ on\ column\ injection} \times 100\%$$

4. Results and discussion

4.1. Sensory comparison of neat liquors and SAFE-DIST isolates

Sensory difference testing was conducted to assess whether perceived aroma differences existed between the original liquor products (neat liquors) and their respective SAFE-DIST isolates. Out of 24 judges, 7–11

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of them, depending on the specific liquors, were able to correctly identify the odd samples, which is still well below the minimal level of correct response of 13 needed for there to be a significant difference ($p \leq 0.05$) (Table 1) (Meilgaard & Civille, 2007). Thus, the results of sensory difference testing demonstrated that the overall perceived aroma profiles of the original liquor products and their respective SAFE-DIST isolates did not differ ($p \leq 0.05$). Panelists had less correct responses for the 3 clear liquors which were aged in pottery compared to the 3 brown liquors which were aged in oak barrels (Table 1). Even though there was no chance for visual bias (sample appearance was masked), there appeared to have been a context effect in the case of the brown liquors. That is, panelists found it easier to detect differences when 2 neat (brown) liquors were presented with 1 SAFE-DIST isolate. However, effect was not observed when 2 SAFE-DIST isolates were presented with 1 neat liquor. To account for such context effects, it is appropriate that both serving orders be considered in the final statistical analysis (Meilgaard & Civille, 2007).

Table 1. Triangle difference test comparison of perceived aroma attributes of neat versus SAFE-DIST isolates for selected liquors.

Liquor		**Panel response (no. correct/no. total assessments)**			
		(2 SAFE – 1 Neat)[a]	**(2 Neat – 1 SAFE)**[b]	**Total**	**Sign. Diff.**[c]
Clear liquor products	MT[d]	4/12	3/12	7/24	N
	GJGJ[e]	5/12	7/12	12/24	N
	YPJZ[f]	5/12	3/12	8/24	N
Brown liquor products	DJT[g]	4/12	6/12	10/24	N
	EWW[h]	2/12	10/12	12/24	N
	AER[i]	4/12	7/12	11/24	N

a 2 SAFE-1 Neat: Triangle test of 1 original liquor and 2 of their corresponding SAFE distillate.

b 2 Neat-1 SAFE: Triangle test of 2 original liquor and 1 of their corresponding SAFE distillate.

c Significant difference: N = not significantly different ($p \leq 0.05$).

d MT: Moutai.

e GJGJ : Gu Jing Gong Jiu.

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f
YPJZ: Yi Pin Jing Zhi.

g
DJT: Don Julio Tequila.

h
EWW: Evan Williams Kentucky Bourbon Whiskey.

i
AER: Appleton Estate Jamaica Rum.

4.2. Volatile profile comparison of neat liquors and SAFE-DIST isolates

An additional volatile profile comparison was made between neat liquors and their respective SAFE-DIST isolates. Eight compounds (2-methyl-1-propanol, 3-methy-1-butanol, ethyl hexanoate, tetramethylpyrazine, ethyl 2-phenylacetate, hexanoic acid, 2-phenylethanol and octanoic acid) possessing different functional groups (including 2 fusel alcohols, 2 fatty acids, 2 esters, 1 pyrazine and 1 phenolic compound) and different molecular weights and volatilities, were selected for analysis (Table 2). Among these 8 compounds, 2-methyl-1-propanol, 3-methy-1-butanol and ethyl hexanoate differed by no more than 2% between the original liquors and their respective SAFE-DIST isolates. For all 6 liquors, the difference between the peak areas of ethyl 2-phenylacetate and 2-phenylethanol were below 5% between the original liquor products and their SAFE-DIST isolates. However, for the 2 fatty acids analyzed, the differences between the original liquor products and their SAFE isolates were more significant for the brown liquors than for the clear liquors.

Table 2. GC-FID peak area[a] comparison for selected volatile components between various liquor products (neat) and their respective distillates prepared by direct solvent-assisted flavor evaporation (SAFE-DIST).

	2-Methy-1-propanol	3-Methy-1-butanol	Ethyl hexanoate	Tetramethyl-pyrazine	Ethyl-2-phenylacetate	Hexanoic acid	2-Phenyl ethanol	Octanoic acid
MT Neat	156	400	22.6	12.1	162	11.0	19.4	1.22
MT SAFE	156	402	22.5	8.12	154	10.8	18.7	1.22
% Diff.	0.00130	0.613	0.224	33.0[c]	4.89[b]	1.66	3.48[b]	0.0872
GJGJ Neat	101	288	314	–	–	777	2.98	1.22

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	2-Methy-1-propanol	3-Methy-1-butanol	Ethyl hexanoate	Tetramethyl-pyrazine	Ethyl-2-phenylacetate	Hexanoic acid	2-Phenyl ethanol	Octanoic acid
GJGJ SAFE	101	283	293	–	–	759	2.86	1.22
% Diff.	0.303	2.00	1.64	–	–	2.31	4.12	0.0872
YPJZ Neat	172	794	–	–	44.6	136	40.2	1.87
YPJZ SAFE	171	794	–	–	43.5	134	39.0	1.79
% Diff.	0.919	0.0604	–	–	2.34[c]	1.47	3.04	0.0425
EWW Neat	224	1123	–	–	–	8.56	26.2	2.01
EWW SAFE	224	1121	–	–	–	7.31	25.0	0.964
% Diff.	0.0639	0.127	–	–	–	14.6[c]	4.36	52.0[c]
DJT Neat	403	736	–	–	–	2.49	6.88	2.71
DJT SAFE	395	729	–	–	–	2.44	6.78	2.70
% Diff.	1.99[b]	0.923	–	–	–	2.07	1.39	0.614
AER Neat	113	314	–	–	–	–	2.71	1.82
AER SAFE	113	314	–	–	–	–	2.70	1.04
% Diff.	0.168	0.0996	–	–	–	–	0.614	42.8[c]

a

Average peak areas determined by GC-FID (n = 3).

b

Peak areas differed between SAFE-DIST isolates and original (neat) liquor ($p < 0.05$).

c

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Peak areas differed between SAFE-DIST isolates and original (neat) liquor ($p < 0.01$).

4.3. AEDA comparison of neat liquors and SAFE-DIST isolates

One clear liquor (MT) and one brown liquor (EWW) were selected for further study to evaluate potential differences with respect to the predominant odorants in neat liquors and their respective SAFE-DIST isolates. A combined total of 61 odorants were detected through application of GC–MS-O analysis and AEDA of the MT and EWW original liquors and their respective SAFE-DIST isolates, with 52 odorants detected in MT and 37 detected in EWW (Table 3).

Table 3. Comparative AEDA results for potent odorants ($\log_3 FD \geq 2$) in Moutai (MT) and Evan Williams Bourbon Whiskey (EWW) neat liquors and their respective distillates prepared by direct solvent-assisted flavor evaporation (SAFE-DIST).

No.	Compound	Odor description	Retention Index		Log_3FD factor MT[a]				Log_3FD factor EWW[b]	
			Stabilwax	Rxi-5ms	Stabilwax		Rxi-5ms		Neat	SAFE-DIST
					Neat	SAFE-DIST	Neat	SAFE-DIST		
1	2-methylpropanal	malty	<800	<700	4	4	4	4	–	–
2	acetal	fruity, painty	938	741	7	7	5	5	4	4
3a,b	2-/3-methylbutanal	malty	947	<700	6	6	6	6	1	1
4	ethyl propanoate	fruity	972	720	5	5	4	4	4	4
5	ethyl 2-methylpropanoate	fruity, berry	979	768	9	9	8	8	–	–
6	2,3-butanedione	buttery, creamy	993	<700	2	2	2	2	–	–
7	2-methylpropyl acetate	solvent	1015	792	2	2	3	3	–	–
8	ethyl butanoate	fruity	1042	806	8	8	6	6	4	4
9	ethyl 2-methylbutanoate	fruity, berry	1057	857	10	10	8	8	4	4
10	ethyl 3-methylbutanoate	blueberry	1075	862	8	8	6	6	3	3
11	ethyl pentanoate	fruity, berry	1142	902	6	6	5	5	1	1
12	ethyl 2-methylpentanoate*	fruity, berry	1142	944	6	6	4	4	–	–
13	propyl 2-methylbutanoate	fruity	1183	–	4	4	–	–	–	–

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No.	Compound	Odor description	Retention Index		Log_3FD factor MT[a]				Log_3FD factor EWW[b]	
			Stabilwax	Rxi-5ms	Stabilwax		Rxi-5ms		Neat	SAFE-DIST
					Neat	SAFE-DIST	Neat	SAFE-DIST		
14	ethyl 4-methylpentanoate	fruity, berry	1195	971	6	6	4	4	0 [c]	0
15a,b	2-/3-methyl-1-butanol	malty	1213	749	4	4	2	2	5	5
16	ethyl hexanoate	fruity	1240	999	5	5	4	4	4	4
17	furfuryl ether *	ether like	1296	831	3	3	2	2	–	–
18	2-methyl-3-furanthiol	beefy, vitamin	–	872	–	–	5	5	–	–
19	1-octen-3-one*	mushroom	1312	1029	1	1	1	1	3	3
20	ethyl heptanoate	fruity	1341	1099	1	1	0	0	3	3
21	dimethyl trisulfide	cabbage	1385	969	5	5	3	3	5	5
22	ethyl cyclohexylcarboxylate *	fruity, berry	1430	1135	4	4	3	3	0	0
23	ethyl octanoate	waxy, plastic	1440	1197	3	3	2	2	3	3
24	2-furfurylthiol*	meaty, coffee	1447	912	1	1	4	4	–	–
25	2-ethyl-3,5-dimethylpyrazine	roasted	1463	1063	5	5	3	3	–	–
26	methional*	potato	1475	907	–	–	–	–	4	4
27	(*E*)-2-nonenal*	metallic, fatty	1518	1150	3	2	–	–	3	3
28	unknown	berry	1544	962	4	4	1	1	–	–
29	2-methylpropanoic acid	cheesy	1582	843	1	1	2	2	–	–
30	butanoic acid	cheesy	1637	872	3	3	0	0	–	–
31	phenylacetaldehyde	rosy	1663	1032	3	3	2	2	–	–
32a,b	2-/3-methyl butanoic acid	cheesy, sweaty	1676	–	4	3	–	–	1	1
33	2-methyl-3-(methyldithio) furan	vitamin	1681	1174	5	5	3	3	–	–
34	3-methyl-2,4-nonadione*	hay, fatty	1695	–	2	2	3	3	–	–
35	pentanoic acid	cheesy	1747	–	2	2	–	–	–	–

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No.	Compound	Odor description	Retention Index		Log_3FD factor MT[a]				Log_3FD factor EWW[b]	
			Stabilwax	Rxi-5ms	Stabilwax		Rxi-5ms		Neat	SAFE-DIST
					Neat	SAFE-DIST	Neat	SAFE-DIST		
36	dimethyltetrasulfide	cabbage	1760	1214	4	3	2	2	–	–
37	ethyl phenylacetate	rosy	1801	1247	3	3	4	4	–	–
38	2-phenylethyl acetate	fruity, rosy	1829	–	1	1	–	–	4	4
39	β-damascenone	applesauce	1837	1384	5	5	5	5	4	4
40	geraniol	citrus	1859	–	3	2	–	–	2	2
41	guaiacol	smoky	1884	1091	3	2	0	0	5	5
42	ethyl 3-phenylpropanoate	fruity, grape	1898	1349	5	5	4	4	2	2
43	2-phenylethanol	rosy	1933	1130	5	5	1	1	5	5
44	β-ionone	floral	1954	–	1	1	–	–	2	2
45	4-ethylguaiacol	smoky, clove	2055	1281	1	1	–	–	3	3
46	γ-nonalactone	peachy	2062	1380	3	2	0	0	1	1
47	*p*-cresol	animal barn	2107	1106	3	3	0	0	–	–
48	unknown	hay, sweet	2131	–	–	–	–	–	2	2
49	ethyl cinnamate	rosy, fruity	2159	–	1	1	–	–	2	2
50	*bis*(2-methyl-3-furyl) disulfide	meaty	2178	1526	2	2	–	–	–	–
51	eugenol	spicy, clove	2188	–	–	–	–	–	6	6
52	4-ethylphenol	manure, stable	2205	–	3	3	–	–	0	0
53	3-hydroxy-4,5-dimethyl-furan-2(5*H*)-one (sotolone)	curry, spicy	2233	–	5	5	–	–	7	7
54	unknown	plastic	2266	–	3	2	–	–	1	1
55	syringol	bacon, smoky	2305	–	1	1	–	–	4	4
56	unknown	herbal	2386	–	1	1			2	2
57	phenylacetic acid	rosy	2603	1287	3	3	0	0	–	–
58	vanillin	vanilla	2624	–	1	2	–	–	7	5

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No.	Compound	Odor description	Retention Index		Log_3FD factor MT[a]				Log_3FD factor EWW[b]	
			Stabilwax	Rxi-5ms	Stabilwax		Rxi-5ms		Neat	SAFE-DIST
					Neat	SAFE-DIST	Neat	SAFE-DIST		
59	3-phenylpropanoic acid	sweaty, rosy	2657	1349	2	2	–	–	–	–
60	unknown	cabbage	–	1057	–	–	3	3	–	–

* Compound was tentatively identified by matching its RI and odor properties with authentic standard compound.

a AEDA was conducted on Stabilwax and Rxi-5ms GC columns.

b AEDA was conducted on Stabilwax GC column.

c Indicates that compound was detected in the concentrated aroma extract only.

As indicated in Table 3, 55 compounds were either positively or tentatively identified, while 5 were unidentified (unknown). The identified compounds included 18 esters (nos. 4, 5, 7–14, 16, 20, 22, 23, 37, 38, 42 and 49); 7 acids (nos. 29, 30, 32a, 32b, 35, 57 and 59); 7 sulfur-containing compounds (nos. 18, 21, 24, 26, 33, 36 and 50); 4 ketones (nos. 6, 19, 39 and 44); 4 aldehydes (nos. 1, 3a/3b, 27); 7 phenols/guaiacols (nos. 41, 45, 47, 51, 52, 55 and 58); 4 alcohols (nos. 15a/15b, 40 and 43); 1 pyrazine (no. 25); 1 lactone (no. 46); 1 acetal (no. 2), 1 furanone (no. 53) and 1 ether (no. 17).

As expected, MT and EWW differ with respect to their aroma profiles. Whiskey derives much of its aroma components a result of aging in oak barrels. Many of these oak-derived volatiles contribute to the unique aroma profile of whiskey; e.g., guaiacol, 4-ethylguaiacol, eugenol, syringol and vanillin (Conner, Paterson, & Piggott, 1993). On the other hand, MT is aged in pottery, an essentially inert container. There are of course other differences between MT and EWW caused by use of different raw materials, fermentation, distillation, etc. in their production.

All of the odorants identified in EWW have been previously reported in the literature (Lahne., 2010, Poisson and Schieberle, 2008). Most of the odorants in MT were previously reported (Chen et al., 2013, Fan et al., 2012, Zhu et al., 2007). However, several additional odorants are reported for the first time in this study, including 2-methyl-3-furanthiol (no. 18), ethyl cyclohexylcarboxylate (no. 22), (*E*)-2-nonenal (no. 27), 2-methyl-3-(methyldithio)furan (no. 33); dimethyltetrasulfide (no. 36) and *bis*(2-methyl-3-furyl) disulfide (no. 50).

The results AEDA are depicted as log_3flavor dilution (FD) factors to allow for a clearer comparison between each of the neat liquors and their respective SAFE-DIST isolates. For MT, AEDA results were identical for

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the neat liquor and the SAFE-DIST isolate, with the exception of only 5 odorants (nos. 27, 37, 47, 55 and 59) which differed by no more than 1 log_3FD factor between the two extracts. The AEDA results for EWW showed a similar trend, with only 1 odorant (vanillin, no. 59) being detected at a higher log3 FD factor in the neat liquor. In all cases where differences were observed, the odorants were detected at slightly lower log_3FD factors in the SAFE-DIST isolates, possibly due to poor recovery of these compounds by SAFE.

4.4. Stability of selected sulfur compounds in MT

Seven sulfur compounds (nos. 18, 21, 24, 26, 33, 36 and 50) were detected in MT. Noteworthy among these were 2-methyl-3-furanthiol (MFT, no. 18), 2-methyl-3-(methyldithio) furan (MFT-MT no. 33) and *bis*(2-methyl-3-furyl) disulfide (MFT-MFT, no. 50), which have not been previously reported in MT and which possess intense savory (meaty and vitamin-like) aromas. It is known that MFT-MFT is formed by dimerization of MFT and, similarly, MFT-MT can be formed by reaction of MFT with methanethiol (Mottram and Whitfield, 1995, Weerawatanakorn et al., 2015). It has also been reported that MFT can be oxidized during storage to its dimer MFT-MFT (Hofmann et al., 1996). MFT-MFT when exposed to an elevated temperature can be converted back to MFT, while MFT-MT is thermally stable (Belitz, Grosch, & Schieberle, 2009).

Based on the above observations, there was some concern over whether MFT (no. 18) was an artifact formed by the analysis conditions used in this study. Cold (cryo) spliltess injection using a PTV inlet was employed for GC–MS-O. During injection the temperature of the inlet was ramped from –50 °C to 250 °C. This means that for a brief period of time the volatile components were exposed to elevated temperatures as they were transferred from the inlet into the GC column. For this reason, cool on-column GC-O analysis was conducted to confirm the results obtained by cold-splitless GC–MS-O analysis (Table 4). By comparing the results from cool on-column GC-O analysis against the results of cold-splitless GC–MS-O analysis, it was found that 2-methyl-3-furanthiol (MFT), which has a FD factor of 243 by cold-splitless injection, was not detected at all by cool on-column GC-O analysis. This is an indication that MFT might be a thermally generated artifact. Therefore, an additional study was conducted to examine the relative stabilities of MFT-MFT and MFT-MT during typical GC analysis conditions.

Table 4. Thermal degradation of selected odor-important disulfides as a function of GC injection method.

No.			Cool on-column	Cold-splitless	Hot splitless (inlet temperature)		
					200 °C	250 °C	300 °C
50	MFT-MFT[a]	Peak area ratio (mean ± STD)	6.658 ± 0.022	5.544 ± 0.027	5.294 ± 0.033	4.547 ± 0.038	3.848 ± 0.038
		Degradation (%)	0.0	16.7	20.5	31.7	42.2
33	MFT-MT[b]	Peak area ratio (mean ± STD)	0.5737 ± 0.0022	0.5745 ± 0.0012	0.5748 ± 0.0025	0.5733 ± 0.0015	0.5730 ± 0.0037

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No.		Cool on-column	Cold-splitless	Hot splitless (inlet temperature)		
				200 °C	250 °C	300 °C
	Degradation (%)	0.0	0.0	0.0	0.0	0.0

a
MFTMFT: *bis*(2-methyl-3-furyl) disulphide.

b
MFT-MT: 2-methyl-3-(methyldithio) furan.

The stabilities of MFT-MT and MFT-MFT and their potential to form MFT were tested under various injection scenarios, including hot splitless (200, 250 and 300 °C), cold splitless and cool on-column injection, using the same PTV inlet (modified for each injection method) and under the same GC–MS conditions. The results showed that MFT-MT was stable under all injection conditions tested, but MFT-MFT stability was highly dependent upon the injection conditions used (Table 4). MFT-MFT was most stable when cool on-column injection was used, but appreciable degradation (>16%) occurred under either cold or hot splitless injection. Furthermore, as expected, hot splitless injection resulted in the greatest degradation of MFT-MFT, with around 42% degradation occurring when the inlet temperature was 300 °C. Even though on-column is an ideal method to avoid the degradation of MFT-MFT with subsequent formation of MFT, it is not a viable option for comparison between the neat liquor and its respective SAFE isolate because this method has little tolerance for “dirty” aroma extracts.

4.5. Quantitative comparison of selected aroma

Twenty-four odorants were selected for quantitation based on SIDA to provide a more accurate comparison between two neat liquors (MT and EWW) and their respective SAFE isolates (Table 5). Compounds chosen for analysis included 3 Strecker aldehydes (nos. 1, 3a and 3b), 11 esters (nos. 8, 10, 11, 16, 20, 23, 37, 42, 61, 62 and 63), 7 acids (nos. 29, 30, 35, 57, 64, 65 and 66), 1 alcohol (no. 43) and 3 semi-volatile components (nos. 53, 58 and 67). The quantitation results showed that highly volatile components like Strecker aldehydes, short chain fatty acids and low molecular weight ethyl esters had high SAFE-DIST recoveries from 98.1 to 100% for both MT and EWW. However, the recovery ethyl decanoate, ethyl dodecanoate and ethyl hexadecanoate were only 61.0% and 59.2% (for EWW) and 20.78% (for MT), respectively. These higher molecular weight esters are not very odor-active and thus do not contribute significantly to the overall aroma profile to the liquor products, which explains why even though their recoveries by SAFE were poor, the perceived aromas of the resulting SAFE-DIST isolates were still not significantly different from their respective neat liquors. However, these components may contribute to the mouthfeel of a liquor product and might induce a taste difference if eliminated. The recoveries of other semi-volatile components like vanillin and syringaldehyde (for EEW) were only 17.14% and 5.24%, respectively, which agrees with the AEDA results for vanillin, since the $\log_3$FD factor differed by 2 dilutions, roughly one ninth in concentration.

Table 5. Concentration comparison of selected potent odorants in original neat liquors of Moutai (MT) and Evan Williams Bourbon Whiskey (EWW) and their respective distillates prepared by direct solvent-assisted

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flavor evaporation (SAFE-DIST).

No.	Compound	Neat MT[a]	MT SAFE-DIST[a]	% recovery	Neat EWW[a]	EWW SAFE-DIST[a]	% recovery
1	2-methylpropanal	26.12 ± 0.66	25.74 ± 0.40	98.6	0.920 ± 0.022	0.911 ± 0.013	99.0
3a	2-methylbutanal	17.70 ± 0.17	17.67 ± 0.29	99.8	0.308 ± 0.017	0.3051 ± 0.0041	100
3b	3-methybutanal	37.11 ± 0.49	37.10 ± 0.46	100	0.3869 ± 0.0061	0.3799 ± 0.0078	98.2
8	ethyl butanoate	57.70 ± 0.41	57.36 ± 0.36	99.4	–	–	–
11	ethyl pentanoate	4.561 ± 0.034	4.539 ± 0.037	99.6	–	–	–
10	ethyl 3-methylbutanoate	11.32 ± 0.057	11.32 ± 0.038	99.9	–	–	–
16	ethyl hexanoate	17.11 ± 0.026	17.11 ± 0.083	100	2.691 ± 0.020	2.684 ± 0.015	99.6
20	ethyl heptanoate	1.250 ± 0.011	1.235 ± 0.013	98.4	–	–	–
23	ethyl octanoate	2.010 ± 0.015	2.005 ± 0.0054	99.5	10.36 ± 0.064	10.32 ± 0.085	99.6
61	ethyl decanoate	–	–	–	12.09 ± 0.15	7.38 ± 0.12	61.0
62	ethyl dodecanoate	–	–	–	5.61 ± 0.19	3.316 ± 0.046	59.2
63	ethyl hexadecanoate	19.25 ± 0.078	3.999 ± 0.041	20.8	–	–	–
37	ethyl phenylacetate	5.420 ± 0.011	5.357 ± 0.031	98.9	–	–	–
42	ethyl 3-phenylpropionate	38.64 ± 0.76	38.55 ± 0.27	99.8	–	–	–
64	acetic acid	7258 ± 0.033	7254 ± 0.031	99.9	2.499 ± 0.064	2.475 ± 0.041	99.2
65	propanoic acid	1229 ± 3.6	1229 ± 2.6	100	–	–	–
30	butyric acid	35.18 ± 0.25	35.06 ± 0.26	99.7	–	–	–
29	2-methylpropanoic acid	20.89 ± 0.19	20.50 ± 0.04	98.1	–	–	–
35	pentanoic acid	4.383 ± 0.037	4.384 ± 0.0096	100	–	–	–
66	hexanoic acid	12.19 ± 0.037	12.10 ± 0.088	99.3	–	–	–
57	phenylacetic acid	20.44 ± 0.027	14.20 ± 0.016	69.5	–	–	–
43	2-phenylethanol	20.77 ± 0.15	19.11 ± 0.10	92.0	28.97 ± 0.12	27.05 ± 0.19	93.4
58	vanillin	–	–	–	2.802 ± 0.015	0.4819 ± 0.0018	17.1
67	syringaldehyde	–	–	–	9.170 ± 0.045	0.4775 ± 0.0025	5.23
53	sotolon	0.0962 ± 0.0010[b] 0.097 ± 0.015[c]	0.08478 ± 0.00050	88.2	–	–	–

a

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Average concentration (mg/L)±standard deviation (n=3).

b

Concentration determined by addition of isotope solution to the neat liquor followed by direct solvent extraction and fractionation without SAFE.

c

Concentration determined by addition of isotope solution to the neat liquor followed by direct solvent extraction, SAFE distillation and fractionation.

The quantitation results of sotolon by three different methods were in agreement in the case of MT, since the results of SIDA without SAFE and with SAFE (isotope added before SAFE distillation) were nearly identical. Therefore, sotolon is not an artifact but an odor-active component in this liquor. Results also show the recovery of sotolon by SAFE distillation was above 88%, which could explain why the $\log_3$FD factors of sotolon in the SAFE distillate and original liquor did not differ.

5. Conclusions

The proposed streamlined approach for quantitation of odor-active components in distilled liquors was evaluated by various methods, including sensory testing, semi-quantitative analysis (GC–MS-O AEDA) and advanced quantitation (SIDA). Among the selected 6 liquors examined, the aroma profiles did not differ between original liquors and those of SAFE-DIST. Assessed by AEDA, within selected clear and brown liquor samples, most potent odor-active components had the same FD factor in the extract of liquor products and their SAFE isolates. According to the SIDA results of odor-active components before and after SAFE, the difference in concentrations of most odor-active components between original liquors and their SAFE isolates were negligible, including Strecker aldehydes, short chain fatty acids and ethyl esters from butanoic acid to octanoic acid. Semi-volatile components, e.g. long chain ethyl esters, vanillin, and syringaldehyde, were not well isolated by the stream-lined approach due to poor recoveries by SAFE distillation. Thus, for the quantitation of semi-volatile compounds the isotopes have to be added before SAFE distillation to guarantee accurate quantitation results. Compared with the standard way to accomplish the quantitation of compounds using combined SAFE and SIDA, the “addition-extraction-adjustment” procedure to achieve reasonable isotope-to-target analyte ratios is significantly shortened by the proposed streamlined approach by allowing the researcher to quantitate more odorants without additional SAFE operations as long as the SAFE isolate is properly prepared and preserved. Furthermore, the direct SAFE-DIST isolate can be used for both identification and quantitation purposes which would not be feasible by following the traditional procedures. Thus this proposed approach not only allows quantitation of odor-active components with significant less time, effort, materials (especially important in the case of rare source materials) and isotopes. This approach could potentially be applied for the study of the flavor chemistry of wine, beer, fruit pulps and other aqueous food products.

Acknowledgement

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Declaration of Competing Interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

The following are the Supplementary data to this article:

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Supplementary data 1.

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510 0_ | ‡**a** PMC
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856 40 | ‡**u** https://www.ncbi.nlm.nih.gov/pmc/journals/3708/
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	J01.576.423.850.100
Unique ID	D005504
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D005504
Annotation	general only; prefer /anal with specific food item
Scope Note	Measurement and evaluation of the components of substances to be taken as FOOD.
Entry Version	FOOD ANAL
Entry Term(s)	Analysis, Food
See Also	Food Quality
Date Established	1966/01/01
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362 1 Began with volume 1 (30 March 2019).
520 2 "Food Chemistry X publishes papers dealing with the advancement of the chemistry and biochemistry of foods or the analytical methods/approach used."
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264    _1    $aWashington, D.C. :$bAmerican Chemical Society,$c1983.

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504          $aIncludes bibliographies and index.

650    _0    $aMaillard reaction$vCongresses.

650    _0    $aFood industry and trade$vCongresses.

700    1_    $aWaller, George R.

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710    2_    $aAmerican Chemical Society.$bDivision of Carbohydrate Chemistry.

830    _0    $aACS symposium series ;$v215.

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245 04 The Maillard reaction in foods and nutrition / $c George R. Waller, editor, Milton S. Feather, editor.
260 Washington, D.C. : $b American Chemical Society, $c 1983.
300 xii, 585 pages : $b illustrations ; $c 24 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
490 1 ACS symposium series, $x 0097-6157 ; $v 215
500 "Based on a symposium jointly sponsored by the Divisions of Agricultural and Food Chemistry, and Carbohydrate Chemistry at the 183rd meeting of the American Chemical Society, Las Vegas, Nevada, March 28-April 2, 1982."
504 Includes bibliographical references and index.
505 0 Seventy years of the Maillard reaction / Sin'itiro Kawamura -- A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation / Mitsuo Namiki and Tateki Hayashi -- Analytical use of fluorescence-producing reactions of lipid- and carbohydrate-derived carbonyl groups with amine end groups of polyamide powder / W.L. Porter, E.D. Black, A.M. Drolet, and J.G. Kapsalis -- Strecker degradation products from (1-^{13}C)-D-glucose and glycine / Tomas Nyhammar, Kjell Olsson, and Per-Åke Pernemalm -- Nitrite interactions in model Maillard browning systems / Gerald F. Russell --

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540 Current Copyright Fee: GBP36.92 $c 0. $5 Uk
520 Abstract: Various aspects of the Maillard reaction (a non-enzymatic reaction that gives food its flavor and color during frying, roasting, and baking) are discussed for food scientists and nutritionists in 29 technical papers. The papers are organized into 7 sections, covering: historical development; chemical aspects (6 papers); flavors, tastes, and odors of cooked foods (6 papers); food technology aspects (3 papers); nutritional aspects, with emphasis on lysine losses (6 papers); in vivo Maillard reactions (2 papers); and toxicological aspects, with emphasis on mutagenproduction (5 papers). A literature review of the sensory properties of almost 450 Maillard reaction products is included. (wz).
650 0 Maillard reaction $v Congresses.
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650 2 Food Handling $0 (DNLM)D005511
650 2 Food Technology $0 (DNLM)D005524
650 2 Nutritional Physiological Phenomena $0 (DNLM)D009747
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650 7 Maillard reaction. $2 fast $0 (OCoLC)fst01006114
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655 2 Congress $0 (DNLM)D016423
655 7 Conference papers and proceedings. $2 fast $0 (OCoLC)fst01423772
655 7 Conference papers and proceedings. $2 lcgft
655 7 Actes de congrès. $2 rvmgf $0 (CaQQLa)RVMGF-000001049
700 1 Waller, George R.
700 1 Feather, Milton S., $d 1936-
710 2 American Chemical Society. $b Division of Agricultural and Food Chemistry.
710 2 American Chemical Society. $b Division of Carbohydrate Chemistry.
776 08 $i Online version: $t Maillard reaction in foods and nutrition. $d Washington, D.C. : American Chemical Society, 1983 $w (OCoLC)706868785
830 0 ACS symposium series ; $v 215. $x 0097-6157
856 41 $u http://pubs.acs.org/doi/book/10.1021/bk-1983-0215 $z Rutgers restricted $z Full text available from ACS Symposium Series (1974-2012)

Attachment 27a

MARC Bibliographic Record

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250           $aRevised edition.
264    _1     $aCleveland :$bCRC Press,$c[1975]
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337           $aunmediated$bn$2rdamedia
338           $avolume$bnc$2rdacarrier
504           $aIncludes bibliographical references and index.
650    _0     $aSoyfoods.
700    1_     $aCowan, John Charles,$d1911-
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OCLC 1288343 **No holdings in XXX - 353 other holdings**

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650 6 Soja (Aliment) $0 (CaQQLa)201-0062195
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653 00 sojabonen
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653 00 sojaproducten

653 00 soyabean products
653 10 Nutrients
653 10 Voedingsstoffen
700 1 Cowan, J. C. $q (John Charles), $d 1911-1991, $e author.
776 08 $i Online version: $a Wolf, Walter James, 1927- $t Soybeans as a food source. $b Rev. ed. $d Cleveland : CRC Press, [1975] $w (OCoLC)698047625

Attachment 27c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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450 __ |**w** nne |**a** Soybean as food

550 __ |**w** g |**a** Food

550 __ |**w** g |**a** Soybean products

670 __ |**a** Work cat.: Shurtleff, W. Bibliography of soy ice creams ... c1988: |**b** CIP galley (soyfoods)

670 __ |**a** LC data base, 6/8/88 |**b** (soyfood, soyfoods)

670 __ |**a** Shurtleff, W. Thes. for SOYA |**b** (soyfoods: BT Soy products; NT Soyfoods, Fermented, Soyfoods, Nonfermented)

670 __ |**a** Hennepin |**b** (soybean as food)

670 __ |**a** Mindell, E. Earl Mindell's soy miracle, c1995 |**b** (soy foods)

675 __ |**a** Bender. Dict. nutrit. food technol.; |**a** IAC; |**a** Web. 3; |**a** Kirk-Othmer encyc. chem.

906 __ |**t** 9437 |**u** te05 |**v** 0

952 __ |**a** Made for existing bibliographic records

953 __ |**a** xx00 |**b** td04

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Case No.: IPR2023-00207
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Amino Acid Composition of an Organic Brown Rice Protein Concentrate and Isolate Compared to Soy and Whey Concentrates and Isolates

by Douglas S. Kalman

Nutrition/Endocrinology Department, Miami Research Associates, 6141 Sunset Drive, Suite 301, Miami, FL 33143, USA

Foods **2014**, *3*(3), 394-402; https://doi.org/10.3390/foods3030394

Received: 18 March 2014 / Revised: 9 June 2014 / Accepted: 19 June 2014 / Published: 30 June 2014

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Abstract

A protein concentrate (Oryzatein-80™) and a protein isolate (Oryzatein-90™) from organic whole-grain brown rice were analyzed for their amino acid composition. Two samples from different batches of Oryzatein-90™ and one sample of Oryzatein-80™ were provided by Axiom Foods (Los Angeles, CA, USA). Preparation and analysis was carried out by Covance Laboratories (Madison, WI, USA). After hydrolysis in 6-N hydrochloric acid for 24 h at approximately 110 °C and further chemical stabilization, samples were analyzed by HPLC after pre-injection derivitization. Total amino acid content of both the isolate and the concentrate was approximately 78% by weight with 36% essential amino acids and 18% branched-chain amino acids. These results are similar to the profiles of raw and cooked brown rice except in the case of glutamic acid which was 3% lower in the isolate and concentrate. The amino acid content and profile of the Oryzatein-90™ isolate was similar to published values for soy protein isolate but the total, essential, and branched-chain amino acid content of whey protein isolate was 20%, 39% and 33% greater, respectively, than that of Oryzatein-90™. These results provide a valuable addition to the nutrient database of protein isolates and concentrates from cereal grains. **View Full-Text**

Keywords: **brown rice**; **supplemental protein**; **essential amino acids**; **branched-chain amino acids**; **muscle protein synthesis**

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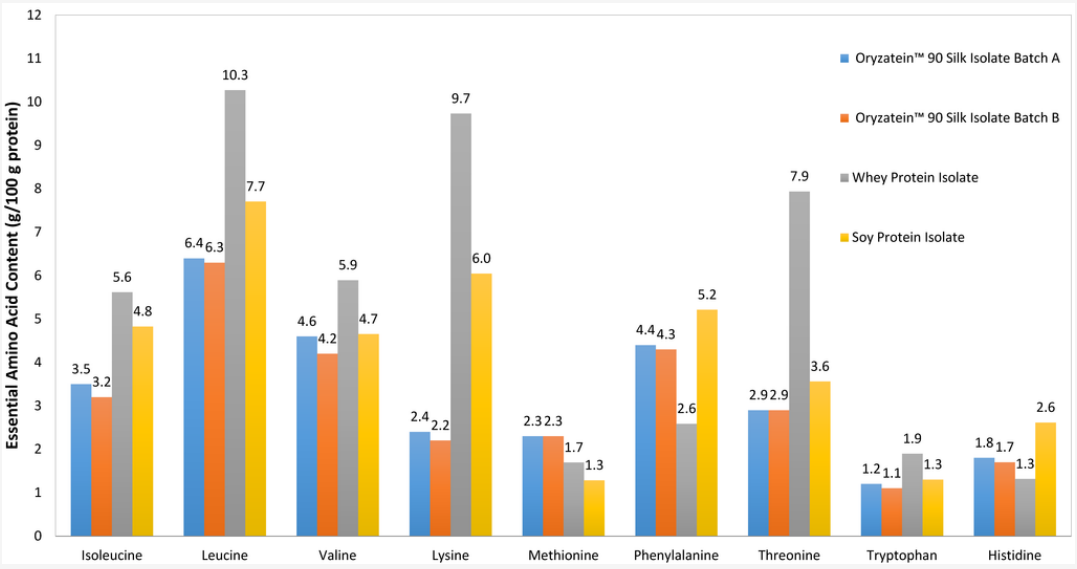


Figure 1

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210 10 |**a** Foods |**2** dnlm
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300 __ |**a** 1 online resource
310 __ |**a** Quarterly

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362 1_ |**a** Began with: Volume 1, issue 1 (December 2012).
515 __ |**a** Numbering from table of contents page.
515 __ |**a** Volume 1 complete in one issue.
588 __ |**a** Description based on: Volume 1, issue 1 (December 2012); title from banner (journal home page, viewed Aug. 15, 2014).
588 __ |**a** Latest issue consulted: Volume 5, issue 3 (September 2016) (viewed Nov. 29, 2016).
650 _0 |**a** Food |**v** Periodicals.
650 _0 |**a** Nutrition |**v** Periodicals.
655 _0 |**a** Electronic journals.
710 2_ |**a** Natural Science Collection
856 40 |**u** https://search.proquest.com/publication/2032399 |**z** Full text available from Natural Science Collection: 01/01/2012 to 12/31/2018
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245 10 $aBailey's industrial oil & fats products.

246 30 $aIndustrial oil & fats products

246 3_ $aBailey's industrial oil and fats products

250 $aSixth edition /$bedited by Fereidoon Shahidi.

264 _1 $aHoboken, N.J. :$bJohn Wiley & Sons,$c[2005]

264 _4 $c©2005

300 $a6 volumes :$billustrations ;$c24 cm

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246 30 Industrial oil & fat products
246 3 Bailey's industrial oil and fat products
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Attachment 30a

MARC Bibliographic Record

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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362    1_  $aBegan with Apr. 1, 1953 issue.$zCf. OCLC.
500        $aDescription based on: Vol. 25, no. 1 (Jan.-Feb. 1977); title from cover.
500        $a"An American Chemical Society publication."
500        $aPlace of publication varies.
530        $aAlso available on the Internet.
530        $aAlso available on microfiche and microfim.
510    2_  $aBiological abstracts$x0006-3169
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510 2_ $aVETDOC

550 $aVols. for 1953-<54> published by the American Chemical Society; <Jan./Feb. 1976-> by the Books and Journals Division, American Chemical Society.

650 _0 $aAgricultural chemistry$vPeriodicals.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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650 _2 $aChemistry, Agricultural$vperiodicals.

710 2_ $aAmerican Chemical Society.

710 2_ $aAmerican Chemical Society.$bBooks and Journals Division.

776 1_ $tJournal of agriculture and food chemistry$x0021-8561$w(OCoLC)07385304

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Case No.: IPR2023-00207
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260 $aWashington, DC :$bAmerican Chemical Society,$c1953-

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321 $aBiweekly,$b2002-2011

321 $aFrequency varies,$b1953-2001

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338 $aonline resource$bcr$2rdacarrier

362 1_ $aPrint began with vol. 1, no. 1 (Apr. 1953).

500 $aRefereed/Peer-reviewed

525 $aSome issues accompanied by supplementary materials or supporting information.

588 $aDescription based on print version record, (ACS Publications, viewed Aug. 29, 2006); title from contents page.

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U.S. Patent No. 11,224,241

588 $aLatest issue consulted: Volume 69, issue 43 (November 3, 2021), (ACS Publications, viewed Nov. 9, 2021).

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Attachment 30b

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Continuing Resources

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321 Monthly, $b <Dec. 1990-2001
321 Bimonthly, $b Jan./Feb. 1960-Nov./Dec. 1989
321 Monthly, $b 1955-1959
321 Biweekly, $b Apr. 1, 1953-<Dec. 22, 1954
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 v. 1- Apr. 1, 1953-

510 2 Chemical abstracts $x 0009-2258
525 Some issues <1978-Jan. 1995 accompanied by supplementary material on microfiche; Feb. 1995-<Feb. 1998, by supporting information on microfiche; current (1995-) supporting information also available online.
530 Also issued on microfiche, microfilm, and online.
550 Volumes for 1953-<54 published by the American Chemical Society; <Jan./Feb. 1976- by the Books and Journals Division, American Chemical Society.
650 0 Agricultural chemistry $v Periodicals.
650 0 Food $x Analysis $v Periodicals.
650 0 Plants $x Nutrition $v Periodicals.
650 2 Chemistry, Agricultural. $0 (DNLM)D002622
650 2 Food-Processing Industry. $0 (DNLM)D005525
650 6 Chimie agricole $v Périodiques.
650 6 Aliments $x Analyse $v Périodiques.
650 6 Plantes $x Nutrition $v Périodiques.
650 7 58.34 food technology. $0 (NL-LeOCL)07760671X $2 bcl
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655 2 Periodical. $0 (DNLM)D020492
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710 2 American Chemical Society. $b Books and Journals Division.
776 08 $i Online version: $t Journal of agricultural and food chemistry (Online) $x 1520-5118 $w (DLC)sn 98004593 $w (OCoLC)39281168
850 CPT $a DLC $a DNAL $a DNGA $a ICRL $a InU $a NN $a NRU $a NcRS $a OrU-S
856 41 $u http://pubs.acs.org/journals/jafcau/index.html

Attachment 31a

The Wayback Machine - https://web.archive.org/web/20170519192635/http://www2.llu.lv:80/research_conf/proceedings.htm



RESEARCH FOR RURAL DEVELOPMENT 2019

17 - 19 May, 2017

Publication Ethics and Malpractice Statement

PROCEEDINGS OF CONFERENCES

- **Annual 22nd ISC Research for Rural Development 2016 Volume 1**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 22nd ISC Research for Rural Development 2016 Volume 2**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 21st ISC Research for Rural Development 2015 Volume 1**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 21st ISC Research for Rural Development 2015 Volume 2**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 20th ISC Research for Rural Development 2014 Volume 1**
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- **Annual 20th ISC Research for Rural Development 2014 Volume 2**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 19th ISC Research for Rural Development 2013 Volume 1**
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- **Annual 19th ISC Research for Rural Development 2013 Volume 2**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 18th ISC Research for Rural Development 2012 Volume 1**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 18th ISC Research for Rural Development 2012 Volume 2**
  ISSN 1691-4031 (print), ISSN 2255-923X (online)
- **Annual 17th ISC Research for Rural Development 2011 Volume 1**
  ISSN 1691-4031 (print)
- **Annual 17th ISC Research for Rural Development 2011 Volume 2**
  ISSN 1691-4031 (print)

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U.S. Patent No. 11,224,241

- **Annual 16th ISC Research for Rural Development 2010 Volume 1**
  ISSN 1691-4031 (print)
- **Annual 16th ISC Research for Rural Development 2010 Volume 2**
  ISSN 1691-4031 (print)
- **Annual 15th ISC Research for Rural Development 2009**
  ISSN 1691-4031 (print)
- **Annual 14th ISC Research for Rural Development 2008**
  ISSN 1691-4031 (print)
- **Annual 13th ISC Research for Rural Development 2007**
  ISSN 1691-4031 (print)
- **Annual 12th ISC Research for Rural Development 2006**
  ISBN 9984-784-14-2 (print)
- **Annual 11th ISC Research for Rural Development 2005**
  ISBN 9984-784-01-0 (print)

HISTORY OF CONFERENCES IN PHOTOS

- **22nd International scientific conference**
  "Research for Rural Development 2016" (Pictures - pdf file)
- **21th International scientific conference**
  "Research for Rural Development 2015" (PowerPoint Show)
- **Conference "Research for Rural Development"**
  historical overview in figures and pictures 1995 - 2014 (PowerPoint Show)
- **20th International scientific conference**
  "Research for Rural Development 2014" (PowerPoint Show)
- **19th International scientific conference**
  "Research for Rural Development 2013" (Pictures - pdf file)
- **18th International scientific conference**
  "Research for Rural Development 2012" (Pictures - pdf file)
- **17th International scientific conference**
  "Research for Rural Development 2011" (In Pictures)
- **16th International scientific conference**
  "Research for Rural Development 2010" (PowerPoint Show)
- **Conference "Research for Rural Development"**
  historical overview in figures and pictures (PowerPoint Show)
- **Home pages from 2003 - 2009 year**
- **15th International scientific conference**
  "Research for Rural Development 2009" (PowerPoint Show)

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

- **14th International scientific conference "Research for Rural Development 2008"**
- **13th International scientific conference "Research for Rural Development 2007"**
- **12th International scientific conference "Research for Rural Development 2006"**
- **11th International scientific conference "Research for Rural Development 2005"**
- **10th International scientific conference "Research for Rural Development 2004"**
- **9th International scientific conference "Research for Rural Development 2003"**

Attachment 31b

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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PERIODICAL OR NEWSPAPER

Research for rural development : annual ... international scientific conference ...

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310	__	|**a** Annual
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Attachment 31c

OCLC 778564079 **No holdings in XXX - 20 other holdings**

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362 1 Began in 2001.
550 Proceedings of the annual International Scientific Conference "Research for Rural Development."
515 Each vol. in two parts.
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588 Latest issue consulted: 17th (2011).
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711 2 International Scientific Conference "Research for Rural Development."

Attachment 31d

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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LC control no.: sh 85002455
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HEADING: Agriculture Study and teaching

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681 __ |**i** Note under |**a** Agricultural education
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024 7_ |**a** 0000000121518114 |**2** isni |**1** http://isni.org/isni/0000000121518114

024 7_ |**a** D007844 |**2** mesh |**0** http://id.nlm.nih.gov/mesh/D007844

024 7_ |**a** 66eb3e73-d69e-3581-9a23-a73b4c64c8dd |**2** musicb |**1** http://musicbrainz.org/area/66eb3e73-d69e-3581-9a23-a73b4c64c8dd#_

024 7_ |**a** 17476-1 |**2** stw |**0** http://zbw.eu/stw/descriptor/17476-1

024 7_ |**a** concept911 |**2** unescot |**0** http://vocabularies.unesco.org/thesaurus/concept911

024 7_ |**a** 125422131 |**2** viaf |**1** http://www.viaf.org/viaf/125422131

024 7_ |**a** 94259 |**2** ysopai |**0** http://www.yso.fi/onto/yso/p94259

034 __ |**d** 25 |**e** 25 |**f** 57 |**g** 57 |**2** geonames

034 __ |**d** E0250000 |**e** E0250000 |**f** N0570000 |**g** N0570000 |**2** geonames

034 __ |**d** 24.6062 |**e** 24.6062 |**f** 56.8794 |**g** 56.8794 |**2** margaz
034 __ |**d** 20.969 |**e** 28.2434 |**f** 58.0861 |**g** 55.6727 |**2** margaz
034 __ |**d** E0204000 |**e** E0281400 |**f** N0580500 |**g** N0554000 |**2** bound
034 __ |**d** 020.6715 |**e** 028.2415 |**f** 058.0856 |**g** 055.6747 |**2** bound
034 __ |**d** E0250000 |**e** E0250000 |**f** N0570000 |**g** N0570000 |**2** wfbcia
035 __ |**a** (OCoLC)oca00530253
040 __ |**a** DLC |**b** eng |**e** rda |**c** DLC |**d** DLC |**d** DLC-H |**d** DLC-S |**d** DLC |**d** WaU |**d** OCoLC |**d** CSt |**d** WaU
043 __ |**a** e-lv--- |**0** http://id.loc.gov/vocabulary/geographicAreas/e-lv
151 __ |**a** Latvia
368 __ |**b** Country |**b** Independent political entity
368 __ |**b** Countries (sovereign states) |**2** aat
368 __ |**b** Republics |**2** lcsh
368 __ |**b** Nations |**2** aat
368 __ |**b** Nation |**2** thesoz
368 __ |**b** National state |**2** thesoz
368 __ |**b** State |**2** eurovocen
368 __ |**b** State |**2** stw
370 __ |**f** Europe |**2** lcsh
370 __ |**f** Europe, Eastern |**2** lcsh
370 __ |**f** Baltic States |**2** lcsh
370 __ |**f** Former Soviet republics |**2** lcsh
370 __ |**f** European Union countries |**2** lcsh
370 __ |**f** OECD countries |**2** lcsh
451 __ |**a** L.P.S.R.
451 __ |**a** Läti Nõukogude Sotsialistlik Vabariik
451 __ |**a** Latvian S.S.R.
451 __ |**a** Latvian Soviet Socialist Republic
451 __ |**a** Latvian SSR
451 __ |**a** Latvi~~i~~a
451 __ |**a** Latviĭska~~i~~a Respublika
451 __ |**a** Latviĭska~~i~~a Sovetska~~i~~a So~~t~~sialisticheska~~i~~a Respublika
451 __ |**a** Latviĭska~~i~~a SSR
451 __ |**a** Latvija
451 __ |**a** Latvijas Padomju Socialistiska Republika
451 __ |**a** Latvijas PSR
451 __ |**a** Latvijas Republika
451 __ |**a** Laṭviyah
451 __ |**a** Latviyskaya Sovetskaya Sotsialisticheskaya Respublika
451 __ |**a** Leṭland
451 __ |**a** Letònia
451 __ |**a** Lettland

451 __ |**a** Lettonie
451 __ |**a** Łotwa
451 __ |**a** LPSR
451 __ |**a** Repubblica Socialista Sovietica della Lettonia
451 __ |**a** Republic of Latvia
451 __ |**a** Латвия
551 __ |**w** a |**a** Lifl~~i~~andska~~i~~a guberni~~i~~a (Russia)
551 __ |**a** Ostland
667 __ |**a** Includes the old catalog heading: Latvian S.S.R.
667 __ |**a** URIs added to this record for the PCC URI MARC Pilot. Please do not remove or edit the URIs.
667 __ |**a** Non-Latin script reference not evaluated.
670 __ |**a** Its Konstitu~~t~~si~~i~~a ... 1944.
670 __ |**a** Its Svod zakonov Latviĭskoĭ SSR, 1981-
670 __ |**a** Markss, A. Lettland in Wort und Bild, heute, 1983.
670 __ |**a** Levin, D. ‘Im ha-gav el ha-ḳir, 1978: |**b** t.p. (Laṭviyah)
670 __ |**a** Its Traités conclus par la Lettonie avec les pays étrangers, 1920-1925, between 1925 and 1929.
670 __ |**a** Mark, M. Di Yidish-ṿelṭlekhe shul in Leṭland, 1973.
670 __ |**a** Washington Post, 09-07-91: |**b** t.p. (Govt. of Soviet Union recognized independence of Latvia on 09-06-91)
670 __ |**a** Soversh. uprav. narod. khoz. v uslov. formir. rynoch. ėkonomiki, 1990: |**b** t.p. (... Latviĭskoĭ Respubliki)
670 __ |**a** BGN. Foreign names info. bull., Feb. 1992, surrogate: |**b** p. 3 (former name: Latviyskaya Sovetskaya Sotsialisticheskaya Respublika [Russian]; Latvian Soviet Socialist Republic [conventional]: ADM1; new name: Latvia [brief], Republic of [conventional]; Latvija [Latvian short form]; Latvijas Republika [Latvian long form]: PCL1, 57°00′N, 25°00′E)
670 __ |**a** UNESCO thesaurus, via WWW, July 23, 2020 |**b** (Latvia. BT Baltic States; Central and Eastern Europe; European Union. In other languages: French: Lettonie; Russian: Латвия = Latvi~~i~~a; Spanish: Letonia)
670 __ |**a** The world factbook, via WWW, July 23, 2020 |**b** (Latvia. Location: Eastern Europe, bordering the Baltic Sea, between Estonia and Lithuania. Geographic coordinates: 57 00 N, 25 00 E. Conventional long form: Republic of Latvia. Conventional short form: Latvia. Local long form: Latvijas Republika. Local short form: Latvija. Former: Latvian Soviet Socialist Republic)
670 __ |**a** Ėn~~t~~sikl. slovar′, 1890-1904: |**b** v. 17, p. 841-847 (Lifl~~i~~andska~~i~~a guberni~~i~~a)
781 _0 |**z** Latvia
952 __ |**a** RETRO
953 __ |**a** xx00 |**b** ee01

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LC control no.: sh 85115899

LCCN Permalink: https://lccn.loc.gov/sh85115899

HEADING: Rural development

000 01220cz a2200325n 450
001 4765226
005 20120328075118.0
008 860211i| anannbabn |b ana
010 __ |**a** sh 85115899
035 __ |**a** (DLC)sh 85115899
035 __ |**a** (DLC)111911
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** HN49.C6
150 __ |**a** Rural development
450 __ |**a** Community development, Rural
450 __ |**a** Development, Rural
450 __ |**a** Integrated rural development
450 __ |**a** Regional development
450 __ |**w** nne |**a** Rehabilitation, Rural
450 __ |**a** Rural community development
450 __ |**a** Rural development |**x** Citizen participation
450 __ |**w** nne |**a** Rural development |**x** Social aspects
450 __ |**a** Rural economic development
550 __ |**w** g |**a** Agriculture and state
550 __ |**w** g |**a** Community development
550 __ |**w** g |**a** Economic development
550 __ |**w** g |**a** Regional planning
680 __ |**i** Here are entered works on the basic development of rural regions in either underdeveloped countries or in deprived areas of more advanced countries, emphasizing improvements in the standard of living and the active participation of the local populations in the programs set up.
906 __ |**t** 9148 |**u** fk04 |**v** 0

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

953 __ |**a** xx00 |**b** se01

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LC control no.: sh 85133052

LCCN Permalink: https://lccn.loc.gov/sh85133052

HEADING: Teaching

000 01301cz a2200337n 450

001 4781802

005 20120328081510.0

008 860211i| anannbabn |a ana

010 __ |**a** sh 85133052

035 __ |**a** (DLC)sh 85133052

035 __ |**a** (DLC)7828504

035 __ |**a** (DLC)sp 85133052

035 __ |**a** (DLC)419668

040 __ |**a** DLC |**c** DLC |**d** DLC

053 _0 |**a** LB1025 |**b** LB1050.7 |**c** Teaching principles and practice

053 _0 |**a** LB1775 |**b** LB1785 |**c** Teaching as a profession

150 __ |**a** Teaching

360 __ |**i** subdivision |**a** Instruction and study |**i** or |**a** Study and teaching |**i** under subjects, e.g. |**a** Music--Instruction and study; Science--Study and teaching

450 __ |**a** Didactics

450 __ |**a** Instruction

450 __ |**a** Pedagogy

450 __ |**a** School teaching

450 __ |**a** Schoolteaching

550 __ |**a** Education

550 __ |**a** Instructional systems

550 __ |**a** Pedagogical content knowledge

550 __ |**a** Training

680 __ |**i** Here are entered works on the art and methods of teaching. Works on the history and methods of training teachers are entered under |**a** Teachers--Training of. |**i** Works on the study of education as a discipline are entered under |**a** Education--Study and teaching.

681 __ |**i** Notes under |**a** Education--Study and teaching; Teachers--Training of

906 __ |**t** 0913 |**u** tc09 |**v** 1
953 __ |**a** xx00 |**b** td13

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LC control no.: sh 85077784

LCCN Permalink: https://lccn.loc.gov/sh85077784

HEADING: Livestock

000 00842cz a2200325n 450
001 4728457
005 20120326102156.0
008 860211i| anannbabn |b ana
010 __ |**a** sh 85077784
035 __ |**a** (DLC)sh 85077784
035 __ |**a** (DLC)75142
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** SF1 |**b** SF140 |**c** General
150 __ |**a** Livestock
360 __ |**i** headings beginning with the word |**a** Livestock
450 __ |**a** Animal husbandry
450 __ |**a** Farm animals
450 __ |**a** Live stock
450 __ |**a** Stock (Animals)
450 __ |**w** nne |**a** Stock and stock-breeding
550 __ |**w** g |**a** Agriculture
550 __ |**w** g |**a** Animal culture
550 __ |**w** g |**a** Animal industry
550 __ |**w** g |**a** Domestic animals
550 __ |**a** Food animals
550 __ |**a** Herders
550 __ |**a** Range management
550 __ |**a** Rangelands
906 __ |**t** 9533 |**u** te05 |**v** 0
953 __ |**a** xx00 |**b** jb03

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OCLC 898200173 **No holdings in XXX - 37 other holdings**

Books

						Rec Stat		**Entered**	20141208	**Replaced**	20161203172245.3
Type	a	**ELvl**	M	**Srce**	d	**Audn**		**Ctrl**		**Lang**	und
BLvl	m	**Form**	o	**Conf**	0	**Biog**		**MRec**		**Ctry**	xx
		Cont		**GPub**		**LitF**	0	**Indx**	0		
Desc	u	**Ills**		**Fest**	0	**DtSt**	q	**Dates**	18uu, 20uu		

007 c $b r $e n
040 COO $b eng $c COO $d OCLCQ
090 $b
245 00 Farmdoc daily.
260 [Place of publication not identified] : $b University of Illinois at Urbana-Champaign, Department of Agricultural and Consumer Economics
300 1 online resource
336 text $b txt $2 rdacontent
337 computer $b c $2 rdamedia
338 online resource $b cr $2 rdacarrier
500 Title from content provider.
856 40 $3 Full text available from AgEcon $i ssid=ssib019906764; dbcode=JAG; providercode=PRVAYO $u http://ageconsearch.umn.edu/handle/183384

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001	991192453406676
005	20211203184600.0
008	731018c19549999gw mr p 0||| 0ger
022	__ | ‡**a** 0340-2371‡**l** 0340-2371‡**y** 0014-021X‡**y** 0367-0899
030	__ | ‡**a** ERUMAT
035	__ | ‡**9** 0373222
040	__ | ‡**a** DNLM‡**c** DNLM
041	0_ | ‡**a** ger
044	__ | ‡**9** Germany
060	00 | ‡**a** W1‡**b** ER6924
245	00 | ‡**a** Ernährungs-Umschau.
260	__ | ‡**a** Frankfurt am Main.
260	3_ | ‡**a** Frankfurt Am Main :‡**b** Umschau Verlag Breidenstein
300	__ | ‡**a** v.‡**b** illus., ports.
310	__ | ‡**a** Monthly
336	__ | ‡**a** text‡**b** txt‡**2** rdacontent
337	__ | ‡**a** unmediated‡**b** n‡**2** rdamedia
338	__ | ‡**a** volume‡**b** nc‡**2** rdacarrier
362	0_ | ‡**a** Jahrg. [1]- Juni 1954-
500	__ | ‡**a** Vols. for 1992?- Have separately paged section called: Ernahrungslehre und -Praxis.

550 __ | ‡**a** Official organ of the Deutsche Gesellschaft für Ernährung.
650 12 | ‡**a** Nutritional Physiological Phenomena
655 _2 | ‡**a** Periodical
710 2_ | ‡**a** Deutsche Gesellschaft für Ernährung.
740 02 | ‡**a** Ernahrungslehre und -Praxis.
935 __ | ‡**a** (DNLM)119245-nlmdb
995 __ | ‡**a** AUTH‡**b** 19731018‡**c** REV‡**d** 20181116
996 __ | ‡**a** NOTCNSR‡**b** 20000101
999 __ | ‡**a** AUTH
035 __ | ‡**a** (DNLM)E12380000(s)
035 __ | ‡**a** (OCoLC)02916970

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OCLC 2916970 **No holdings in XXX - 20 other holdings**

Continuing Resources

Rec Stat		**Entered**	19770426	**Replaced**	20210115183924.0						
Type	a	**ELvl**		**Srce**	d	**GPub**		**Ctrl**		**Lang**	ger
BLvl	s	**Form**		**Conf**	0	**Freq**	m	**MRec**		**Ctry**	gw
S/L	0	**Orig**		**EntW**		**Regl**	r	**Alph**	b		
Desc		**SrTp**	p	**Cont**		**DtSt**	c	**Dates**	1954 , 9999		

010 sn 85005779
040 COO $b eng $c COO $d SER $d RCS $d AIP $d NSD $d AIP $d CRL $d AGL $d OCLCQ $d NLM $d OCLCQ $d UKMGB $d OCLCF $d OCLCO $d OCLCQ $d OCLCO $d OCLCQ $d OCLCO
012 $j P
016 7 0373222 $2 DNLM
016 7 E12380000 $2 DNLM
016 7 007855061 $2 Uk
019 1065166701 $a 1231032910 $a 1231110872
022 0014-021X $l 0014-021X $y 0340-2341 $2 z
030 ERUMAT
041 0 ger $b eng
042 nsdp
050 4 QP141 $b .E71
060 00 W1 $b ER6924
070 0 389.8 $b Er66
090 $b
210 0 Ernähr.- umsch.
222 0 Ernährungs- Umschau
245 00 Ernährungs- Umschau.
260 Frankfurt am Main, $b Umschau Verlag.
300 $b illustrations $c 30 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
362 0 Jahrg. 1- 1954-
500 Organ der Deutschen Gesellschaft für Ernährung.
510 2 Chemical abstracts $x 0009-2258
546 English summaries.
650 0 Nutrition $v Periodicals.
650 0 Food $v Periodicals.
650 7 Food. $2 fast $0 (OCoLC)fst00930458
650 7 Nutrition. $2 fast $0 (OCoLC)fst01042187
650 12 Nutritional Physiological Phenomena. $0 https://id.nlm.nih.gov/mesh/D009747
655 7 Periodicals. $2 fast $0 (OCoLC)fst01411641
655 2 Periodicals. $0 https://id.nlm.nih.gov/mesh/D020492
710 2 Deutsche Gesellschaft für Ernährung.
776 08 $i Online version: $t Ernährungs- Umschau $w (OCoLC)689304219

Attachment 33c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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LC control no.: sh2008103974
LCCN Permalink: https://lccn.loc.gov/sh2008103974
HEADING: Food Periodicals

000 00456cz a2200169n 450
001 7441920
005 20110729160255.0
008 080211|| anannbabn |n ana
010 __ |**a** sh2008103974
035 __ |**a** (DLC)379540
035 __ |**a** (DLC)sh2008103974
040 __ |**a** DLC |**b** eng |**c** DLC
150 __ |**a** Food |**v** Periodicals
667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: European food research & technology,
906 __ |**t** 8888 |**u** te00 |**v** 0
953 __ |**a** te00

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Attachment 33d

Suchbegriff | Webcode | DOI

Anmelden

EN

Legume flours: Nutritionally important sources of protein and dietary fiber

15.02.2016 | English Articles | Gerhard Jahreis | Melanie Brese | Matthias Leiterer | Ulrich Schäfer | Volker Böhm

Peer-reviewed | Manuscript received: June 12, 2015 | Revision accepted: October 15, 2015

Introduction

Demand is increasing for grain legumes, such as pea, lupin, beans, and lentils. An increase in the content of grain legumes in food would support sustainable agriculture and nutrition, and would be good for health. The use of grain legumes in Europe needs to be developed. This is why both scientists and politicians are attempting to promote human consumption of these valuable foods. One result has been that the area in Germany under cultivation for pea and broad beans doubled in 2015 relative to the previous year.

Highly processed foods are now consumed in large quantities, but have a lower content of protein than conventionally produced foods. “Tasty”, cheap, and energy dense components are deliberately selected for processing and manufacturing, with the consequence that the proportions of carbohydrate and fat increases at the expense of protein. Analysis of the nutrient supply in the USA between 1971 and 2006 found that the protein fraction decreased by 1 energy percent (En%) and the carbohydrate and fat fraction increased by 8 En% [1]. Other studies too have indicated “protein dilution” in highly processed food. This leads to an imbalance, which is evident in increased energy uptake from carbohydrates and fat and decreased energy uptake from proteins. Although the German Nutrition Society (DGE) specifies a reference value of 15 En% from protein, there are demands to increase this to 30 En% [2].

Summary

Vegetarian and vegan nutrition have become fashionable. Some consumers now see themselves as partial vegetarians and greatly restrict their consumption of animal products. This has led to an increase in the demand for alternative sources of protein, in the form of legumes and their processed products. Proteins enhance the feeling of fullness and can contribute to lowering blood pressure. Moreover, plant proteins have a favorable effect on lipid metabolism. For this reason, legume flours are important sources of plant protein.

Flours from soya, lupin, chickpea and green pea have been studied. The highest content of protein was found in soya and lupin, which both contained ca. 35 g protein per 100 g. Lupin flour had the highest content of kernel fiber, corresponding to 35 g/100 g soluble kernel fiber. In addition, lupin and soya flour only had a low content of easily digestible carbohydrates: ca. 8–10 g/100 g.

Cereal flour had a high content of starch and a low content of protein and is used in making bread, cakes and pastries; one possibility would be to replace 10–20% of this with legume flour. The amino acids contained in the legume flour would enhance the nutritional value of the cereal protein.The fat

protein and fiber extracts from legumes.

For ecological and nutritional/physiological reasons, only plant protein should be used to enrich protein relative to fats and carbohydrates. Lupin is an interesting alternative to soya beans in Europe, as it has a high content of protein. Legume flour can be used to enrich bread, cakes or pastries, or meat products. It supports good health and is easy to process.

Keywords: legume flour, pulses, plant protein, dietary fiber, protein quality, fat quality, carotenoids, vitamins, trace elements

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Artikel Fakten

Autor: Gerhard Jahreis, Melanie Brese, Matthias Leiterer, Ulrich Schäfer, Volker Böhm
Datum: 15.02.2016
Seiten: 1

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Possible Mechanism for Involvement of Cysteine in Aroma Production in Wine

Stéphanie Marchand, Gilles de Revel, Joseph Vercauteren, and Alain Bertrand

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Abstract

Under conditions close to those of wine, that is, low pH, aqueous medium, and low temperatures, this work describes *N*-(2-sulfanylethyl)-2-oxopropanamide (**1**), a new intermediate in the formation of 2-acetylthiazole from methylglyoxal and cysteine. **1** was characterized by MS, derivatization MS, and ^{1}H and ^{13}C NMR and was synthesized from 2-sulfanylethanamine and ethyl pyruvate. A formation pathway for 2-acetylthiazole from methylglyoxal and cysteine is proposed, in which **1** is a new intermediate in Maillard-type reactions in systems under mild conditions.

Keywords: *N*-(2-Sulfanylethyl)-2-oxopropanamide; Maillard reaction; cysteine; 2-acetylthiazole; wine aroma

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Commentary: *In Vitro*-Cultured Meat Production

P.D. Edelman, D.C. McFarland, V.A. Mironov, and J.G. Matheny

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Commentary

In Vitro-Cultured Meat Production

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INTRODUCTION

ALTHOUGH MEAT has enjoyed sustained popularity as a foodstuff, consumers have expressed growing concern over some consequences of meat consumption and production. These include nutrition-related diseases, foodborne illnesses, resource use and pollution, and use of farm animals. Here we review the possibility of producing edible animal muscle (i.e., meat) *in vitro*, using tissue-engineering techniques. Such "cultured meat" could enjoy some health and environmental advantages over conventional meat, and the techniques required to produce it are not beyond imagination. To tissue engineers this subject is of interest as cultured meat production is an application of tissue-engineering principles whose technical challenges may be less formidable than those facing many clinical applications.

work or microcarrier beads, and then perfused with a culture medium in a stationary or rotating bioreactor. By introducing a variety of environmental cues, these cells fuse into myotubes, which can then differentiate into myofibers.[2] The resulting myofibers may then be harvested, cooked, and consumed as meat. van Eelen, van Kooten, and Westerhof hold a Dutch patent for this general approach to producing cultured meat.[3] However, Catts and Zurr appear to have been the first to have actually produced meat by this method.[4]

A scaffold-based technique may be appropriate for producing processed (ground, boneless) meats, such as hamburger or sausage. But it is not suitable for producing highly structured meats, such as steaks. To produce these, one would need a more ambitious approach, creating structured muscle tissue as self-organizing constructs[5] or proliferating existing muscle tissue *in vitro*.

The latter technique was employed by Benjaminson, Gilchriest, and Lorenz, the first researchers to have ap-

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CULTURED MEAT PRODUCTION

Most edible animal meat is made of skeletal muscle tissue. The idea that skeletal muscle tissue-engineering techniques could be applied to produce edible meat dates back at least 70 years,[1] but has been seriously pursued by only three groups of researchers. Their efforts can be divided roughly into scaffold-based and self-organizing techniques.

In scaffold-based techniques, embryonic myoblasts or adult skeletal muscle satellite cells are proliferated, attached to a scaffold or carrier such as a collagen mesh-

plied tissue-engineering techniques to meat production.[6] They placed skeletal muscle explants from goldfish (*Carassius auratus*) in diverse culture media for 7 days and observed an increase in surface area between 5.2 and 13.8%. When the explants were placed in a culture containing dissociated *Carassius* skeletal muscle cells, explant surface area increased by 79%.

Explants have the advantage of containing all the cells that make up muscle in their corresponding proportions, thus closely mimicking an *in vivo* structure. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if

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550 __ |**w** g |**a** Cultures (Biology)
550 __ |**a** Organ culture
906 __ |**t** 9642 |**u** te04 |**v** 0
953 __ |**a** xx00 |**b** ta25

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Library of Congress Authorities
URL: http://authorities.loc.gov/
Library of Congress Online Catalog
URL: https://catalog.loc.gov/

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Attachment 36a

Welcome, Guest. MU students, faculty and staff please login for full access.

Protein-protein interaction of soy protein isolate from extrusion processing [electronic resource] / by Angela Chiang.

Language: English

Authors: Chiang, Angela

Publication Information: Columbia, Mo. : University of Missouri-Columbia, 2007.

Publication Date: 2007

Publication Type: eBook

Document Type: eBook

Subject Terms:
Soy proteins
Starch
Extrusion process
Electronic dissertations

Abstract: Abstract: The effects of various extrusions on protein-protein interactions in soy protein isolate were investigated. Soy protein isolate and wheat starch at 9:1 ration were extruded at 35% and 60% moisture content and 124.2, 134 and 140.6ºC product temperature. Dead stop procedure and repeated extrusion were done with 60% moisture content and 124.2ºC product temperature. The protein solubility results show that although product temperatures did not cause significant difference on the amount of soluble proteins, samples between low moisture and high moisture extrusion did. The results of SEM, color analysis and protein solubility test suggested that the physical appearances and chemical bonds were affected by the second extrusion. In all extrusions and the dead stop procedure, the decrease in protein solubility after extrusion was due to the formation of the three

dimensional network of the soy protein isolate polypeptide chains. From all SDS-PAGE tests the results suggested that the extrusion of soy protein isolates modified very little at the molecular level and that the protein subunits did not have any structural changes.

Notes: Thesis advisor: Fu-Hung Hsieh.
Title from title screen of research.pdf file (viewed on May 11, 2009)
The entire dissertation/thesis text is included in the research.pdf file; the official abstract appears in the short.pdf file (which also appears in the research.pdf); a non-technical general description, or public abstract, appears in the public.pdf file.
M.S. University of Missouri-Columbia 2007.
Includes bibliographical references.
Mode of access: World Wide Web.

Other Authors: Hsieh, Fu-hung, thesis advisor

OCLC: 320770785

Online Access: Freely available online

Accession Number: mer.oai.edge.umsystem.folio.ebsco.com.fs00001083.5e5f9579.c795.5853.87f0.bbbb424c860a

Database: MU Libraries Catalog

Location	Call No.	Status
MU Online Library		Available

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Attachment 36b

OCLC 320770785 **No holdings in XXX - 2 other holdings**

Books

						Rec Stat		**Entered**	20090511	**Replaced**	20220928083508.9
Type	a	**ELvl**	3	**Srce**	d	**Audn**	f	**Ctrl**		**Lang**	eng
BLvl	m	**Form**	o	**Conf**	0	**Biog**		**MRec**		**Ctry**	mou
		Cont	bm	**GPub**		**LitF**	0	**Indx**	0		
Desc	a	**Ills**	a	**Fest**	0	**DtSt**	s	**Dates**	2007,		

007 c $b r $d m $e n
040 MUU $b eng $c MUU $d MUU $d OCLCQ $d OCLCF $d OCLCQ $d OCLCO $d OCLCQ
090 $b
100 1 Chiang, Angela.
245 10 Protein-protein interaction of soy protein isolate from extrusion processing / $c by Angela Chiang.
260 Columbia, Mo. : $b University of Missouri-Columbia, $c 2007.
336 text $b txt $2 rdacontent
337 computer $b c $2 rdamedia
338 online resource $b cr $2 rdacarrier
500 The entire dissertation/thesis text is included in the research.pdf file; the official abstract appears in the short.pdf file (which also appears in the research.pdf); a non-technical general description, or public abstract, appears in the public.pdf file.
538 Mode of access: World Wide Web.
500 Title from title screen of research.pdf file (viewed May 11, 2009).
500 Thesis advisor: Fu-Hung Hsieh.
502 $b M.S. $c University of Missouri-Columbia $d 2007
520 3 The effects of various extrusions on protein-protein interactions in soy protein isolate were investigated. Soy protein isolate and wheat starch at 9:1 ration were extruded at 35% and 60% moisture content and 124.2, 134 and 140.6°C product temperature. Dead stop procedure and repeated extrusion were done with 60% moisture content and 124.2°C product temperature. The protein solubility results show that although product temperatures did not cause significant difference on the amount of soluble proteins, samples between low moisture and high moisture extrusion did. The results of SEM, color analysis and protein solubility test suggested that the physical appearances and chemical bonds were affected by the second extrusion. In all extrusions and the dead stop procedure, the decrease in protein solubility after extrusion was due to the formation of the three dimensional network of the soy protein isolate polypeptide chains. From all SDS-PAGE tests the results suggested that the extrusion of soy protein isolates modified very little at the molecular level and that the protein subunits did not have any structural changes.
504 Includes bibliographical references.
650 0 Soy proteins.
650 0 Starch.
650 0 Extrusion process.
650 6 Protéines de soja. $0 (CaQQLa)201-0227080
650 6 Extrusion (Mécanique) $0 (CaQQLa)201-0062280
650 7 extrusion. $2 aat $0 (CStmoGRI)aat300053124
650 7 Extrusion process. $2 fast $0 (OCoLC)fst00919095

650 7 Soy proteins. $2 fast $0 (OCoLC)fst01127465
650 7 Starch. $2 fast $0 (OCoLC)fst01131597
655 2 Academic Dissertation $0 (DNLM)D019478
655 7 Academic theses. $2 fast $0 (OCoLC)fst01726453
655 7 Academic theses. $2 lcgft
655 7 Thèses et écrits académiques. $2 rvmgf $0 (CaQQLa)RVMGF-000001173
700 1 Hsieh, Fu-hung. $4 ths
856 40 $u http://hdl.handle.net/10355/5099 $z Freely available online

Attachment 36c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

LIBRARY OF CONGRESS AUTHORITIES

The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

LC control no.: sh 91003350
LCCN Permalink: https://lccn.loc.gov/sh91003350
HEADING: Soy proteins

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001 4838495
005 20120330074303.0
008 910710i| anannbabn |a ana
010 __ |**a** sh 91003350
035 __ |**a** (DLC)sh 91003350
035 __ |**a** (DLC)185180
040 __ |**a** DLC |**c** DLC |**d** DLC
150 __ |**a** Soy proteins
550 __ |**w** g |**a** Seed proteins
550 __ |**w** g |**a** Soybean products
670 __ |**a** Thes. for SOYA, 1986 |**b** (Soy proteins)
670 __ |**a** LC database, July 5, 1991 |**b** (soy protein)
670 __ |**a** Phone call to W. Shurtleff, July 5, 1991 |**b** (Soy proteins have both food and industrial uses; the most common form for food use is textured soy protein)
670 __ |**a** Kirk-Othmer encyc. chem.
906 __ |**t** 9902 |**u** te07 |**v** 1
952 __ |**a** Made for existing bibliographic records
953 __ |**a** fg04 |**b** ta25

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Attachment 36d

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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LC control no.: sh 85127386
LCCN Permalink: https://lccn.loc.gov/sh85127386
HEADING: Starch

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001 4776272
005 20120328080736.0
008 860211i| anannbabn |a ana
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035 __ |**a** (DLC)sh 85127386
035 __ |**a** (DLC)122957
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** QK898.S67 |**c** Plant physiology
053 _0 |**a** QP702.S75 |**c** Biochemistry
053 _0 |**a** TP248.S7 |**c** Chemical technology
053 _0 |**a** TP415 |**b** TP416 |**c** Food technology
150 __ |**a** Starch
450 __ |**a** Amylum
450 __ |**a** Farinaceous products
450 __ |**a** Potato starch
450 __ |**a** Products, Farinaceous
550 __ |**w** g |**a** Glucans
670 __ |**a** Szejtl, J. Cyclodextrin technology, c1988.
670 __ |**a** MESH.
670 __ |**a** Merck.
906 __ |**t** 8833 |**u** fk04 |**v** 1
952 __ |**a** 0 bib. records to be changed
953 __ |**a** xx00 |**b** ff01

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Attachment 36e

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

LC control no.: sh 85046633
LCCN Permalink: https://lccn.loc.gov/sh85046633
HEADING: Extrusion process

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005 20120323073454.0
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035 __ |**a** (DLC)45079
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** TJ1450
150 __ |**a** Extrusion process
360 __ |**i** subdivision |**a** Extrusion |**i** under individual materials and types of materials, e.g. |**a** Metals--Extrusion
550 __ |**w** g |**a** High energy forming
550 __ |**w** g |**a** Manufacturing processes
550 __ |**w** g |**a** Power presses
906 __ |**t** 64-- |**u** ---- |**v** 0
953 __ |**a** xx00 |**b** ta25

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- Download MARC21

LDR	01972cas a2200589 a 4500
001	998836923406676
005	20220629210146.0
007	cr|cnu|||||||
008	990408c19999999enkmr p 0 0eng d
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022	__ | ‡**a** 1462-2912‡**l** 1462-2912‡**9** PY‡**7** (Print)
022	__ | ‡**a** 1462-2920‡**9** EN‡**7** (Electronic)
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035	__ | ‡**9** 100883692
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042	__ | ‡**a** lcd‡**a** nlmcopyc
044	__ | ‡**9** England
050	14 | ‡**a** QR100‡**b** .E582
060	00 | ‡**a** W1‡**b** EN984UM
210	10 | ‡**a** Environ Microbiol‡**2** DNLM
245	00 | ‡**a** Environmental microbiology.
260	__ | ‡**a** Oxford :‡**b** Blackwell Science,‡**c** 1999-
300	__ | ‡**a** v. :‡**b** ill. ;‡**c** 28 cm.
310	__ | ‡**a** Twelve no. a year‡**b** 2001-
321	__ | ‡**a** Bimonthly‡**b** 1999-2000
336	__ | ‡**a** text‡**b** txt‡**2** rdacontent

337 __ | ‡**a** unmediated‡**b** n‡**2** rdamedia
338 __ | ‡**a** volume‡**b** nc‡**2** rdacarrier
362 0_ | ‡**a** Vol. 1, no. 1 (Feb. 1999)-
500 __ | ‡**a** Title from cover.
510 1_ | ‡**9** 1‡**a** MEDLINE‡**b** v1n1, Feb. 1999-
510 0_ | ‡**9** 1‡**a** PubMed‡**b** v1n1, Feb. 1999-
510 1_ | ‡**a** Index medicus
530 __ | ‡**a** Also issued online.
583 __ | ‡**3** v. 1, no. 1 (1999)‡**c** 20001127‡**f** Notice published‡**l** Printed on acid-free paper that meets the full ANSI standard for Permanent Paper
650 12 | ‡**a** Environmental Microbiology
655 _2 | ‡**a** Periodical
772 0_ | ‡**t** Environmental microbiology reports‡**x** 1758-2229‡**w** (OCoLC)422977816‡**w** (DNLM)101499207
856 4_ | ‡**u** http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1462-2920
911 __ | ‡**a** Electronic and print, print canceled 2017
914 __ | ‡**a** Portico‡**b** Current to 2022 as of 20220627‡**c** ISSN_14622912
935 __ | ‡**a** (DNLM)883692-nlmdb
936 __ | ‡**a** Vol. 1, no. 4 (Aug. 1999) LIC
992 __ | ‡**p** P2‡**e** EF‡**a** 20000525
993 __ | ‡**a** HPN‡**b** 20000609
994 __ | ‡**a** CDN‡**b** 20000609
995 __ | ‡**a** AUTH‡**b** 20000614‡**c** REV‡**d** 20220629
996 __ | ‡**a** CONSER‡**b** 20000101
998 __ | ‡**a** ERMSLC 108652‡**b** 20080708
999 __ | ‡**a** AUTH
035 __ | ‡**a** (OCoLC)41134376

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Attachment 37b

Environmental Microbiology MeSH Descriptor Data 2022

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Environmental Microbiology
Tree Number(s)	H01.158.273.540.274 N06.850.425
Unique ID	D004783
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D004783
Annotation	general or unspecified; prefer specifics
Scope Note	The study of microorganisms living in a variety of environments (air, soil, water, etc.) and their pathogenic relationship to other organisms including man.
Entry Version	ENVIR MICROBIOL
Entry Term(s)	Microbiology, Environmental
NLM Classification #	QW 55
Public MeSH Note	85
History Note	85; was NON MESH before 1985
Date Established	1985/01/01
Date of Entry	1999/01/01
Revision Date	2019/05/24

page delivered in 0.153s

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Attachment 37c

OCLC 41134376 **No holdings in XXX - 104 other holdings**

Continuing Resources

						Rec Stat		**Entered**	19990408	**Replaced**	20220219120411.3
Type	a	**ELvl**		**Srce**	c	**GPub**		**Ctrl**		**Lang**	eng
BLvl	s	**Form**		**Conf**	0	**Freq**	m	**MRec**		**Ctry**	enk
S/L	0	**Orig**		**EntW**		**Regl**	r	**Alph**			
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016 7 100883692 $2 DNLM
016 7 007076025 $2 Uk
019 1000794907 $a 1065210622
022 1462-2912 $l 1462-2912 $2 2
030 ENMIFM
042 pcc
050 00 QR100 $b .E582
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082 04 579.175 $2 21
090 $b
210 10 Environ Microbiol $2 dnlm
210 0 Environ. microbiol. $b (Print)
222 0 Environmental microbiology $b (Print)
245 00 Environmental microbiology.
260 Oxford : $b Blackwell Science, $c 1999-
300 volumes : $b illustrations ; $c 28 cm
310 Monthly, $b <May 2001-
321 Bimonthly, $b <Feb. 1999-
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 Vol. 1, no. 1 (Feb. 1999)-
500 Title from cover.
510 0 Chemical abstracts $x 0009-2258
550 Published by Blackwell for the Society for Applied Microbiology, <May 2001-
588 Latest issue consulted: Vol. 12, issue 11 (Nov. 2010).
650 0 Microbial ecology $v Periodicals.
650 0 Microbial ecology.
650 12 Environmental Microbiology $0 (DNLM)D004783
650 6 Écologie microbienne $0 (CaQQLa)201-0009158 $v Périodiques. $0 (CaQQLa)201-0378307
650 6 Écologie microbienne. $0 (CaQQLa)201-0009158

650 7 Microbial ecology. $2 fast $0 (OCoLC)fst01019484
650 17 Micro-organismen. $2 gtt
650 17 Ecologie. $2 gtt
655 2 Periodical $0 (DNLM)D020492
655 7 Periodicals. $2 fast $0 (OCoLC)fst01411641
710 2 Society for Applied Microbiology.
776 08 $i Online version: $t Environmental microbiology (Online) $x 1462-2920 $w (DLC) 00242312 $w (OCoLC)45260595
850 DLC $a DNLM $a GU
856 41 $3 Journal homepage (includes free access to tables of contents and abstracts) $u http://www.blackwell-science.com/emi
856 41 $u http://firstsearch.oclc.org $z Address for accessing the journal using authorization number and password through OCLC FirstSearch Electronic Collections Online. Subscription to online journal required for access to abstracts and full text
856 41 $u http://firstsearch.oclc.org/journal=1462-2912;screen=info;ECOIP $z Address for accessing the journal from an authorized IP address through OCLC FirstSearch Electronic Collections Online. Subscription to online journal required for access to abstracts and full text
856 41 $u http://www.blackwell-synergy.com/issuelist.asp?journal=emi

Attachment 37d

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

LIBRARY OF CONGRESS AUTHORITIES

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LC control no.: sh 85084757
LCCN Permalink: https://lccn.loc.gov/sh85084757
HEADING: Microbial ecology

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001 4735208
005 20120327093807.0
008 860211i| anannbabn |a ana
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035 __ |**a** (DLC)81893
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450 __ |**a** Environmental microbiology
450 __ |**a** Microorganisms |**x** Ecology
550 __ |**w** g |**a** Ecology
550 __ |**w** g |**a** Microbiology
670 __ |**a** LC data base, 2/4/93 |**b** (Environmental microbiology)
906 __ |**t** 9310 |**u** te04 |**v** 0
953 __ |**a** xx00 |**b** jc01

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Advertisement

International Journal of Food Science & Technology / Volume 44, Issue 9 / p. 1705-1712

Effect of substitution of soy protein isolate on aroma volatiles, chemical composition and sensory quality of wheat cookies

Sobhy M. Mohsen, Hoda H. M. Fadel, M. A. Bekhit, Amr E. Edris, Mohamad Y. S. Ahmed

First published: 10 August 2009
https://doi.org/10.1111/j.1365-2621.2009.01978.x
Citations: 48

Read the full text > About

Summary

Wheat flour was substituted with soy protein isolate (SPI) at levels of 0–20% and its effect on aroma volatiles and quality of cookies was evaluated. The chemical analysis showed that the moisture and protein content increased gradually by increasing the added levels of SPI whereas the carbohydrate and fat levels showed the opposite trend. The sensory characteristics, aroma, taste, crispiness and overall acceptability showed significant ($P < 0.05$) increase of up to 10% SPI substitution compared with cookies free of SPI. The gas chromatographic-mass spectrometric (GC-MS) analysis of aroma volatiles revealed that the sample containing 10% SPI had the highest yield (13.57%) of pyranones, the key odourants of cookies. The results of GC-MS analysis were consistent with those of the aroma sensory analysis of cookies during storage.

Citing Literature

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Supporting Information

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Attachment 38b

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022    ##$a1365-2621
023    ##$a09505423
035    ##$a(OCoLC)42201700
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082    04$a664.005
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(Online)
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260    ##$a[Oxford] : $bBlackwell Science Ltd.
310    ##$aEight no. a year, $b2001-
321    ##$aBimonthly, $b<1996>-2000
362    1#$aPrint began with vol. 22, no. 1 (Feb. 1987).
538    ##$aMode of access: World Wide Web.
550    ##$aPublished for the Institute of Food Science & Technology.
588    ##$aDescription based on: Vol. 31, issue 1 (Feb. 1996); title
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588    ##$aLatest issue consulted: Vol. 39, issue 4 (Apr. 2004),
viewed Apr. 13, 2004.
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655    #7$aPériodique électronique (Descripteur de forme) $2rasuqam
655    #7$aRessource Internet (Descripteur de forme) $2rasuqam
710    2#$aInstitute of Food Science and Technology (Great Britain)
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$w(DLC)66009908
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906    ##$aJOURNAL
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001		11158944
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As nutritious food ingredients, gelatin, soy protein, corn zein, and pea protein have many superior properties making them good and widely used microencapsulation shell materials in food industry. In this chapter, the chemical structures, microencapsulation related properties, such as emulsifying property, film-forming property, gelation property, barrier property, and so on, and the commonly used microencapsulation techniques, such as spray drying, coacervation, gelation, and so on, are reviewed for each protein.

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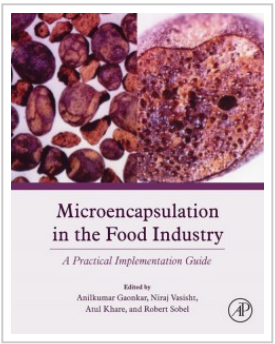
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Microencapsulation in the food industry : a practical implementation guide / edited by Anilkumar G. Gaonkar ... [and three others]



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Abstract: Summary: **Microencapsulation** is being used to deliver everything from improved nutrition to unique consumer sensory experiences. It's rapidly becoming one of **the** most important opportunities for expanding brand potential. **Microencapsulation in the Food Industry**: A Practical Implementation Guide is written for those who see **the** potential benefit of using

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microencapsulation but need practical insight into using **the** technology. With coverage of **the** process technologies, materials, testing, regulatory and even economic insights, this book presents **the** key considerations for putting **microencapsulation** to work. Application examples as well as online access to published and issued patents provide information on freedom to operate, building an intellectual property portfolio, and leveraging ability into potential **in** licensing patents to create produce pipeline. This book bridges **the** gap between fundamental research and application by combining **the** knowledge of new and novel processing techniques, materials and selection, regulatory concerns, testing and evaluation of materials, and application-specific uses of **microencapsulation**.

Notes: Includes bibliographical references and index.
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Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats

Layne E Norton, Gabriel J Wilson, Donald K Layman, Christopher J Moulton & Peter J Garlick

Nutrition & Metabolism **9**, Article number: 67 (2012)

24k Accesses | **76** Citations | **58** Altmetric | Metrics

Abstract

Background

Leucine (Leu) regulates muscle protein synthesis (MPS) producing dose-dependent plasma Leu and MPS responses from free amino acid solutions. This study examined the role of Leu content from dietary proteins in regulation of MPS after complete meals.

Methods

Experiment 1 examined 4 protein sources (wheat, soy, egg, and whey) with different Leu concentrations (6.8, 8.0, 8.8, and 10.9% (w/w), respectively) on the

potential to increase plasma Leu, activate translation factors, and stimulate MPS. Male rats (~250 g) were trained for 14 day to eat 3 meals/day consisting of 16/54/30% of energy from protein, carbohydrates and fats. Rats were killed on d14 either before or 90 min after consuming a 4 g breakfast meal. Experiment 2 compared feeding wheat, whey, and wheat + Leu to determine if supplementing the Leu content of the wheat meal would yield similar anabolic responses as whey.

Results

In Experiment 1, only whey and egg groups increased post-prandial plasma Leu and stimulated MPS above food-deprived controls. Likewise, greater phosphorylation of p70 S6 kinase 1 (S6K1) and 4E binding protein-1 (4E-BP1) occurred in whey and egg groups versus wheat and soy groups. Experiment 2 demonstrated that supplementing wheat with Leu to equalize the Leu content of the meal also equalized the rates of MPS.

Conclusion

These findings demonstrate that Leu content is a critical factor for evaluating the quantity and quality of proteins necessary at a meal for stimulation of MPS.

Background

Leucine (Leu) is an indispensable amino acid with a unique role in initiating protein translation. All amino acids are required as substrates for assembly

of new peptides but Leu serves a second role, particularly in skeletal muscle, as a nutrient signal to initiate muscle protein synthesis (MPS). Leu functions in tandem with hormones including insulin to activate key elements of translation initiation through mTORC1 including the ribosomal protein S6 (rpS6) and the initiation factor eIF4E [1, 2]. With increasing age, the contribution of anabolic hormones to initiate translation declines [3, 4] increasing the importance of Leu as a post-meal anabolic signal (Rieu Nutrition 07; Glynn J. Nutr 2010; Yang Br J Nutr, 2012).

The role of the Leu signal in translation to facilitate assembly of the initiation complex has been studied with free Leu and Leu delivered with indispensable amino acid mixtures [5, 6]. These studies serve to characterize the mechanism of the mTORC1 activation of MPS and established the potential for Leu to generate a post-prandial initiation signal. The role of Leu in triggering translation initiation leads to the assumption that Leu is important in defining the quantity and quality of dietary proteins at a meal necessary to stimulate MPS [7], however this hypothesis has not been well tested.

We hypothesize that the dietary impact of Leu will be greatest during conditions when MPS is down-regulated and the meal content of total protein is limited. To test this hypothesis, we used adult rats to minimize the importance of insulin-stimulated growth signals [4], a short-term food deprivation (12 h) to generate a condition of depressed translation

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initiation [5, 8], and a small meal that was limited in both total energy and protein to optimize the importance of the Leu signal [9]. Specifically, we selected 4 food proteins (wheat gluten, soy protein isolate, egg white protein, and whey protein isolate) representing a Leu range of approximately 6.8% to 10.9% of protein (w/w) that were fed as part of a small breakfast meal providing 20% of total daily energy with protein at 16% of energy and complete profiles of macronutrients and fiber. This study demonstrates that Leu is an important factor of protein quality for translation initiation in skeletal muscle.

Methods

Animals and diets

Male rats (250 ± 12 g) were purchased from Harlan-Teklad (Indianapolis, IN) and maintained at 24°C with a 12-h light:dark cycle and free access to water. The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign.

Rats were trained for 6 day to consume 3 meals/day consisting of a 4 g meal consumed between 07:00 and 07:20 h followed by free access to food from 13:00 to 14:00 and 18:00 to 19:00 [7]. For consistency, all animals were adapted to the meal protocol using the wheat protein diet (Table 1). We have previously tested wheat and whey protein for the meal training and found that adult rats adapt to the meal protocol

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using either protein. After 2 day of meal-training, all rats consumed ~17 g/day of total diet equivalent to ad libitum intake. All diet treatments provided 16/54/30% of energy from protein, carbohydrates and fats, respectively.

Table 1 Composition of diets

Experiment 1 examined post-prandial changes in MPS, plasma Leu, and translation factors in rats fed meals differing in source of protein: wheat (n = 10), soy (n = 10), egg (n = 11), or whey protein (n = 11) (Table 1). All proteins exceeded minimum indispensable amino acid requirements as defined by the National Research Council (NRC) except for wheat gluten that was limiting in lysine (Table 2). Wheat gluten diet was supplemented with lysine to meet NRC requirements and to equal the lysine content of the whey protein isolate (Table 3). A baseline food-deprived control group was also adapted to meal-feeding using the wheat protein diet (n = 10). On d 6 rats were randomly assigned to groups and received their respective treatment diets for 14 day. Previous research evaluated the acute response of MPS to a single meal challenge of wheat versus whey proteins [7]. This study evaluates multiple proteins and uses an extended feeding period to determine if meal responses are maintained over a prolonged period.

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Table 2 Comparison of test diet amino acid compositions with NRC requirements [1,2]

Table 3 Amino acid compositions of protein sources

On d 15, rats were food-deprived for 12 h and then fed their normal treatment 4 g breakfast meal. The food-deprived controls received no breakfast meal. The meals provided 0, 46, 54, 60, and 74 mg of Leu for the food-deprived controls, wheat, soy, egg, and whey groups, respectively. Rats were killed 90 min after consumption of the meal and blood and tissue samples collected. Tissues were then stored at −80°C for later analyses. MPS was measured at 0 and 90-min time-points as described below.

Experiment 2 examined supplementing the wheat gluten meal with Leu to determine if matching Leu contents of the wheat and whey meals would yield similar peak rates of postprandial MPS. Based on findings in Experiment 1 and our previous research [7] demonstrating that the MPS meal response was the same after a single meal or after 14 day feeding, Experiment 2 was performed as a single meal study.

All rats were adapted to meal feeding as described in Experiment 1. After 6 day of adaptation to meal feeding, rats were assigned to treatment groups based on body weight. Animals (n = 5–6 per group) were

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food deprived for 12 h and then groups randomly assigned to either food deprived controls or fed one of three 4 g meals with 16% protein coming from wheat gluten, wheat gluten supplemented with Leu (wheat + Leu) (Ajinomoto, Chicago, IL) (Leu = 18.5 g/kg diet), or whey protein (Table 2). The whey protein and wheat gluten diets were supplemented with glycine (Sigma-Aldrich, St. Lois, MO) to make test meals isonitrogenous and isoenergetic with the wheat + Leu group. Post-meal responses in plasma Leu, were measured at 30, 90, and 135 min after completion of the meal to determine if supplementing free Leu altered the pattern of Leu appearance in the blood. Rats were euthanized and blood and tissues harvested as in Experiment 1. MPS was measured at 0 and 90-min time-points.

Determination of Muscle Protein Synthesis

Protein synthesis was measured in gastrocnemius muscles using the flooding dose method [12]. A 100% enriched L-$^{2}H_5$-phenylalanine solution (150 mmol/L; Cambridge Isotopes, Andover, MA) was administrated at 150 μmol/100 g body weight and injected via tail vein (1 mL/100 g body weight). After 10 min rats were killed by decapitation and hind limbs quickly removed and immersed in an ice-water mixture. Muscles were removed from cooled hind limbs, frozen in liquid N_2, and stored at −80°C.

Frozen muscle was powdered in liquid nitrogen and protein precipitated with cold (4°C) perchloric acid (30 g/L, 1 mL per 50 mg muscle tissue). The resulting supernatant and protein pellet were prepared for gas

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chromatography mass spectroscopy (GC-MS) analyzes as described previously [13, 14]. Enrichment of L-$^{2}H_5$-phenylalanine in the muscle hydrolysate was measured by GC-MS using a 6890 N GC and a 5973 N mass detector (Agilent Technologies Santa Clara, CA). Samples were run under electron impact ionization in splitless mode and phenylethylamine ions with mass-to-charge ratio (m/z) 106 (m + 2) and 109 (m + 5) were monitored for enrichment.

Muscle supernatants were used for determination of intracellular free phenylalanine enrichment. Free amino acids were purified by ion exchange resin solid phase extraction (SPE) using EZ:faastTM amino acid analysis sample testing kit (Phenomenex Inc. Torrance, CA, USA) and $^{2}H_5$-phenylalanine enrichment was determined using a propyl chloroformate derivative with GC-MS monitoring of ions at m/z 206 (m) and 211 (m + 5) [15].

Fractional rates of protein synthesis (MPS) were determined from the rate of incorporation of L-$^{2}H_5$-phenylalanine into total mixed muscle protein as described previously [16]. The time from injection of the metabolic tracer until tissue cooling was recorded as the actual time for L-$^{2}H_5$-phenylalanine incorporation. MPS, defined as the percentage of tissue protein renewed each day, were calculated according to the formula: $MPS = (E_b \times 100)/(E_a \times t)$ where t is the time interval between isotope injection and snap freezing of muscle expressed in days and E_b and E_a are the enrichments of $^{2}H_5$-phenylalanine in

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hydrolyzed tissue protein and in muscle free amino acids, respectively.

Plasma measurements

Plasma was obtained from trunk blood by centrifugation at 1800×*g* for 10 min at 4°C. Plasma insulin concentrations were analyzed using a commercial RIA kit for rat insulin (Linco Research, St. Charles, MO). Plasma glucose (Thermo Fisher Scientific, Middletown, VA) was determined by the glucose oxidase method. Plasma amino acid concentrations were analyzed by HPLC using a Waters 2475 Fluorescence detector [17].

Phosphorylation of 4E-BP1, S6K1, and Akt

Muscle supernatants were subjected to protein immunoblot analyses as described previously [18, 19] using rabbit polyclonal antibodies for 4E-BP1 (Bethyl Labs, Montgomery, TX), S6K1 (Bethyl Labs, Montgomery, TX), and Akt (Cell Signaling, Boston, MA).

Statistical analysis

All data were analyzed by SPSS 15.0 (Chicago, IL) software package for Windows. For Experiment 1, a one-way ANOVA was performed with the treatment groups as the independent variables. For Experiment 2, a one-way ANOVA was performed for MPS, with treatment groups as the independent variables. Other comparisons for Experiment 2 utilized a 3 × 4 (*i.e.* experimental groups: wheat, wheat + Leu, and whey x time: 0, 30, 90, and 135 min) repeated measures

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ANOVA to determine within and between group differences. When a significant overall effect was detected, differences among individual means were assessed using Fisher's LSD post hoc test. Data sets were tested for normal distribution and variance homogeneity using Levene's test. When variances were not homogeneous, means were compared using a Games-Howell test. Significance was set at $P < 0.05$ for all statistical tests. All values are presented as means ± SEM.

Results

Experiment 1

This experiment compared isonitrogenous, isoenergetic meals containing wheat gluten, soy protein isolate, egg white protein, or whey protein isolate as the protein source on the potential to initiate translation and protein synthesis in skeletal muscle. After consumption of a 4 g meal providing 20% of daily energy and containing 16% of energy as protein, plasma Leu increased in egg or whey groups but not in wheat or soy groups. Similar post-prandial patterns occurred for each of the BCAA with whey producing the highest concentrations at 90 min after the meal (*i.e.* whey > egg > soy > wheat) (Table 4).

Table 4 Experiment1: Selected plasma essential amino acid [1] and glucose [2] concentrations 90 min after feeding meals containing wheat, soy, egg, or whey proteins [3-4]

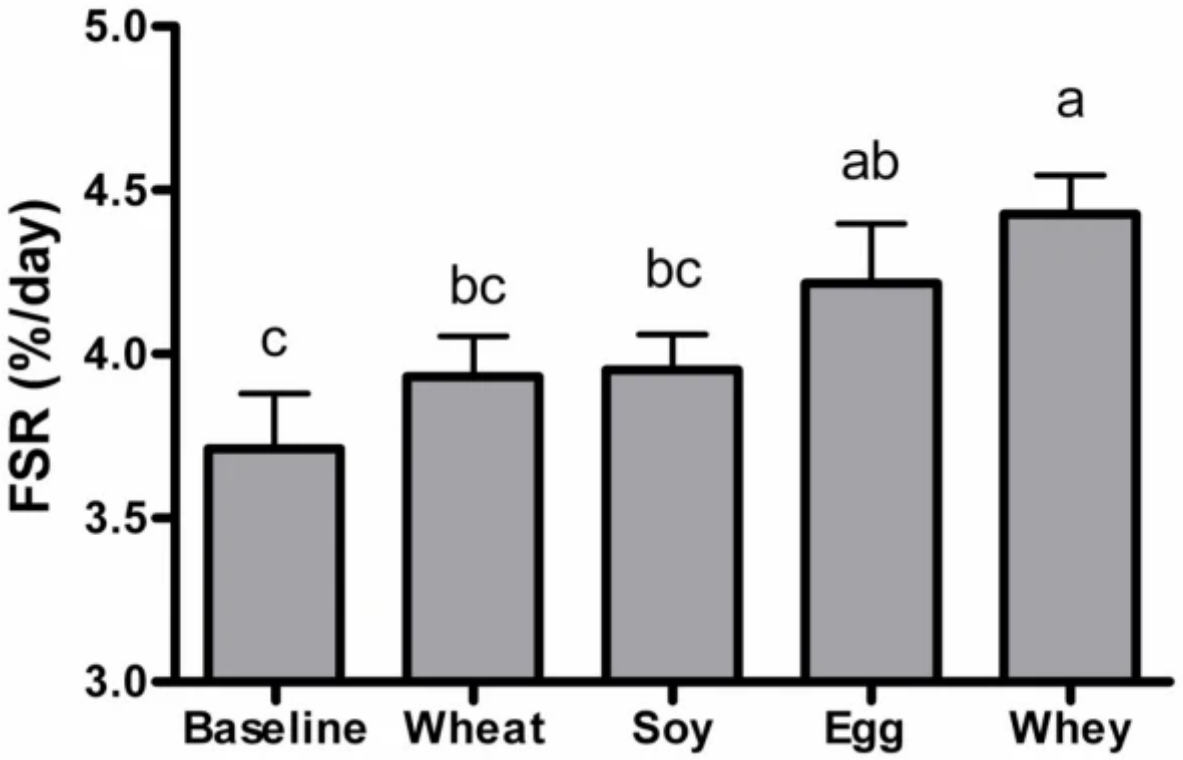
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Other plasma amino acids varied among the groups largely in proportion to the amino acid content of the protein source (Table 4). Lysine was different between soy and whey treatments, with the whey group having the greatest concentration of post-prandial lysine and the soy group having the lowest lysine levels. The plasma concentration for the wheat group reflects the lysine supplementation of the diet. Post-prandial plasma methionine concentrations were increased with the egg treatment while other groups were not different from food deprived controls (Table 3). Plasma threonine was increased after the meal for egg or whey groups with highest concentrations observed in rats consuming the whey protein.

After the meal, MPS increased in the egg or whey groups with the highest value observed for the whey group (Figure 1). The MPS response was consistent with phosphorylation of the mTORC1 signaling targets 4E-BP1 (Figure 2A) and S6K1 (Figure 2B). Phosphorylation of S6K1 increased after egg or whey meals but not wheat or soy with peak values obtained in the whey protein group. 4E-BP1 phosphorylation increased in all groups with whey greater than wheat or soy and egg greater than soy but not different from wheat or whey.

Figure 1

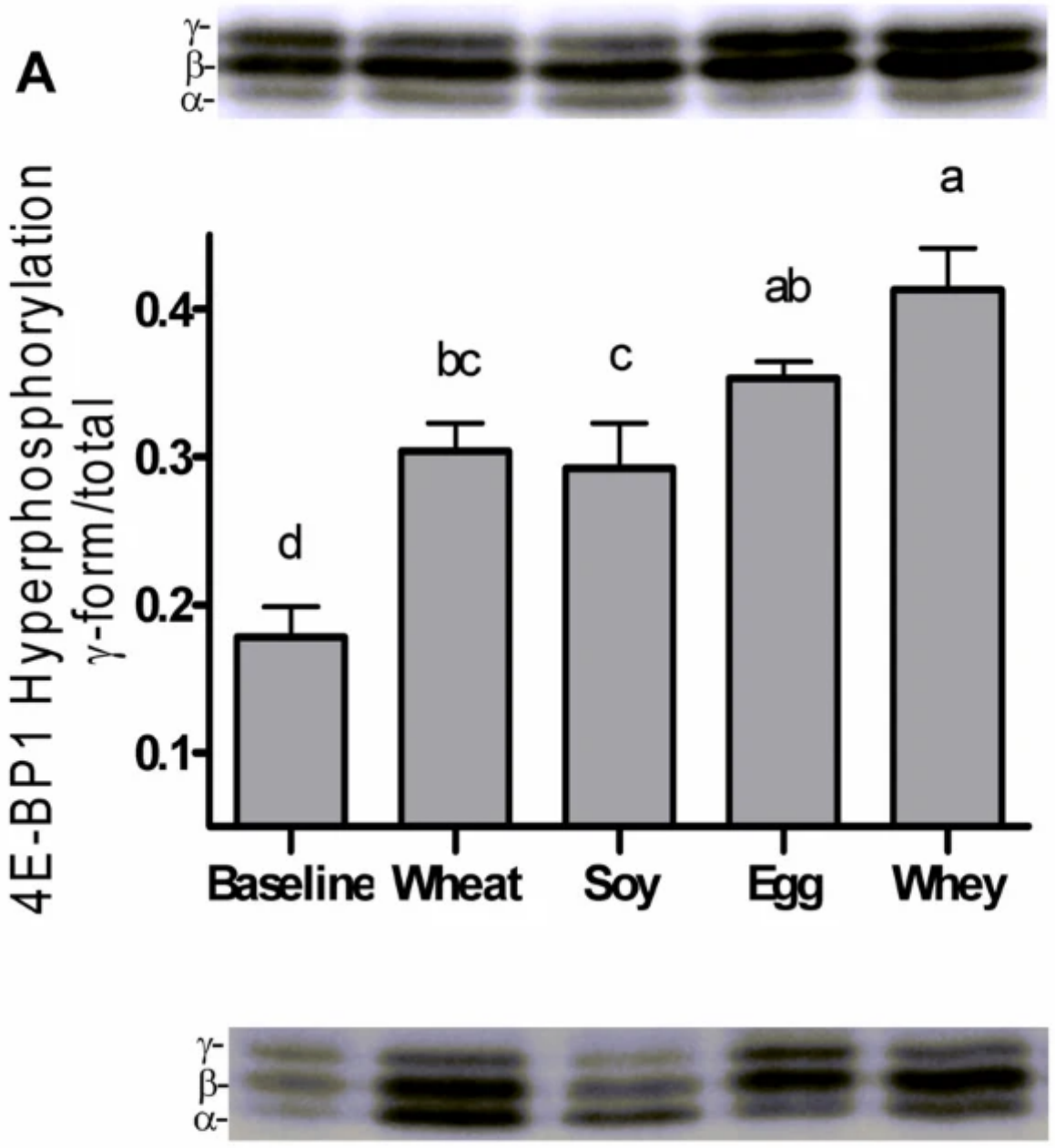
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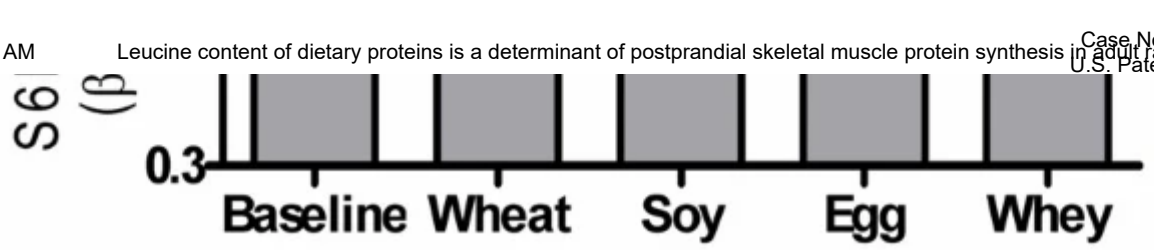


Experiment 1 Muscle Protein Synthesis. Rates of protein synthesis in gastrocnemius muscle of rats after consuming complete meals containing wheat, soy, egg, or whey proteins. Data are means ± SEM; n = 9–10. Labeled means without a common letter differ, $P < 0.05$.

Figure 2

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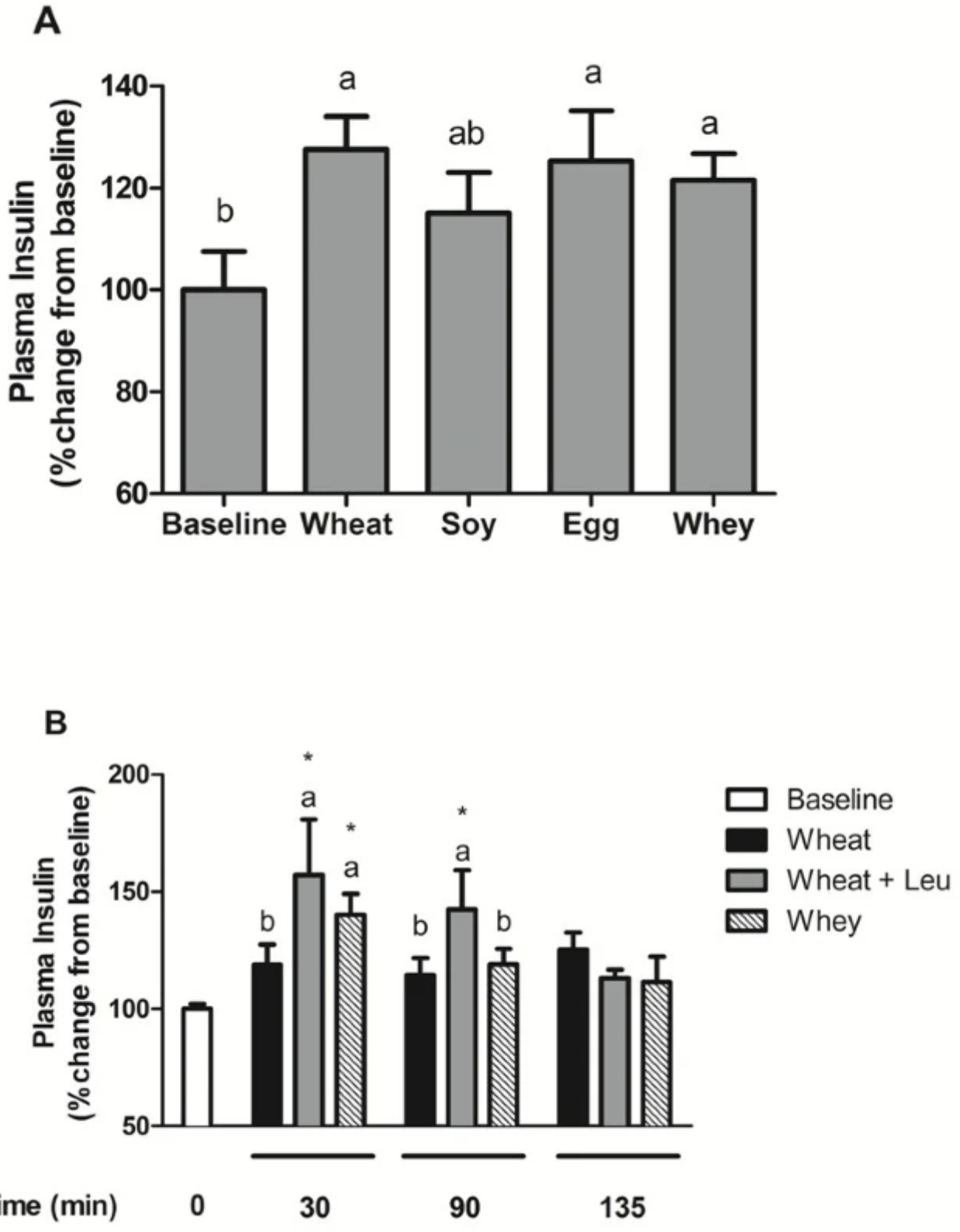


Experiment 1 Translation Factor Activation. Phosphorylation states of 4E-BP1 (**A**) and p70S6K1 (**B**) in gastrocnemius muscle of rats after consuming meals containing wheat, soy, egg, or whey proteins. Data are means ± SEM; n = 9–10. Labeled means without a common letter differ, $P < 0.05$.

Plasma insulin increased above baseline at 90 min after the meal in all groups except soy, which was not different from food-deprived controls (Figure 3). Post-prandial insulin was not significantly different between any of the treatment groups. Similarly, plasma glucose at 90 min was increased above baseline concentrations in all groups except for soy. The glucose concentration for the soy group was not different from baseline or the wheat, egg, or whey groups (Table 4).

Figure 3

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Experiment 1 and 2 Plasma Insulin. Changes in plasma insulin from baseline in rats after **A**) consuming meals containing wheat, soy, egg, or whey proteins or **B**) rats consuming isonitrogenous meals containing wheat,

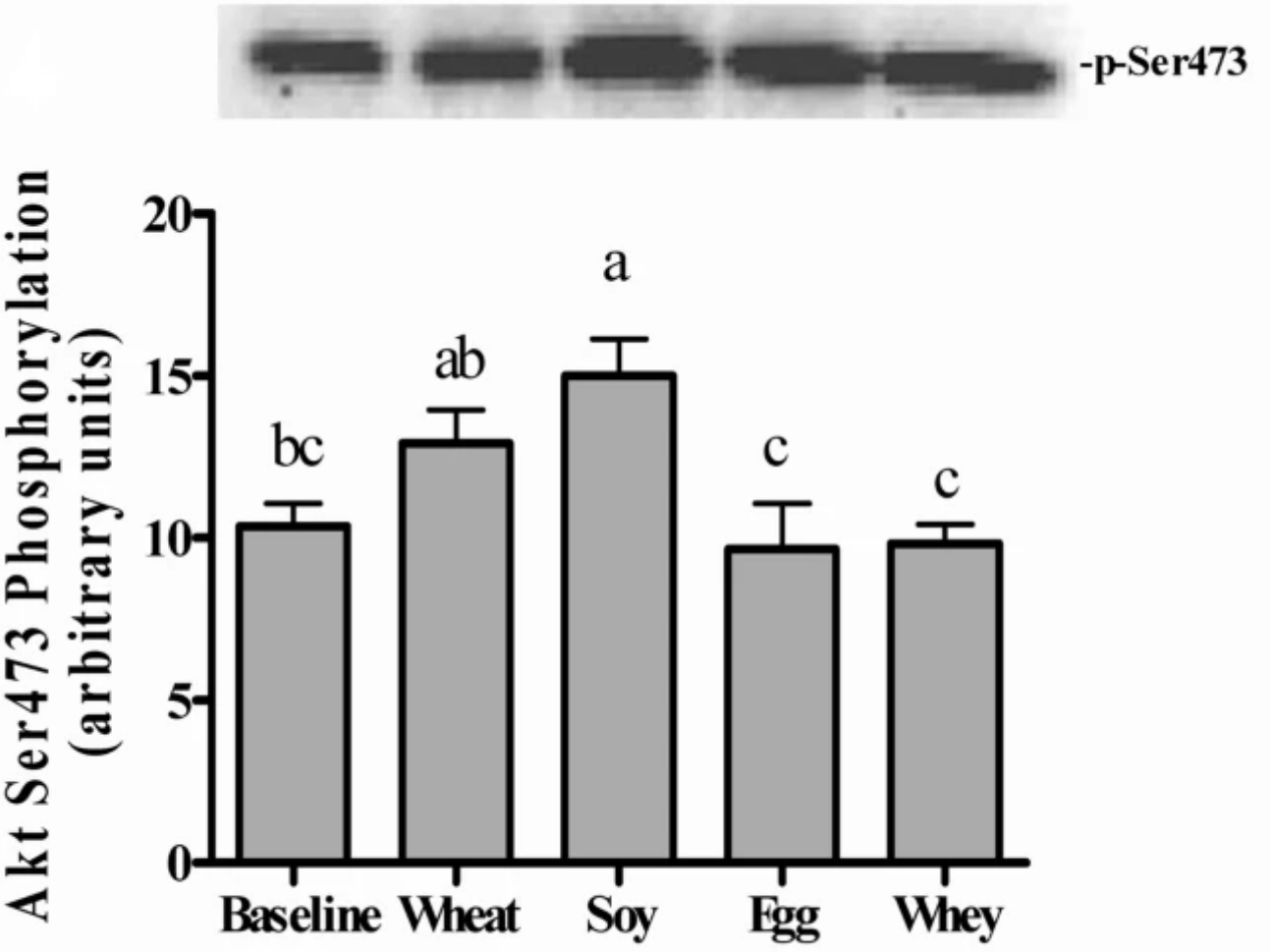
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wheat supplemented with leucine (Wheat + Leu), or whey protein. Data expressed expressed as percent change from baseline and are means ± SEM; n = 5–10.

Akt activation (ie. phosphorylation at Ser473) was increased at 90 min in the soy group compared with food deprived controls rats or the egg or whey groups (Figure 4). The wheat group had intermediate levels of Akt phosphorylation that was not different from baseline or soy groups but greater than egg or whey groups.

Figure 4

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Experiment 1 Akt Phosphorylation.
Phosphorylation states of Akt in gastrocnemius muscle of rats after consuming meals containing wheat, soy, egg, or whey proteins. Data are means ± SEM; n = 9–10. Labeled means without a common letter differ, $P < 0.05$.

Experiment 2

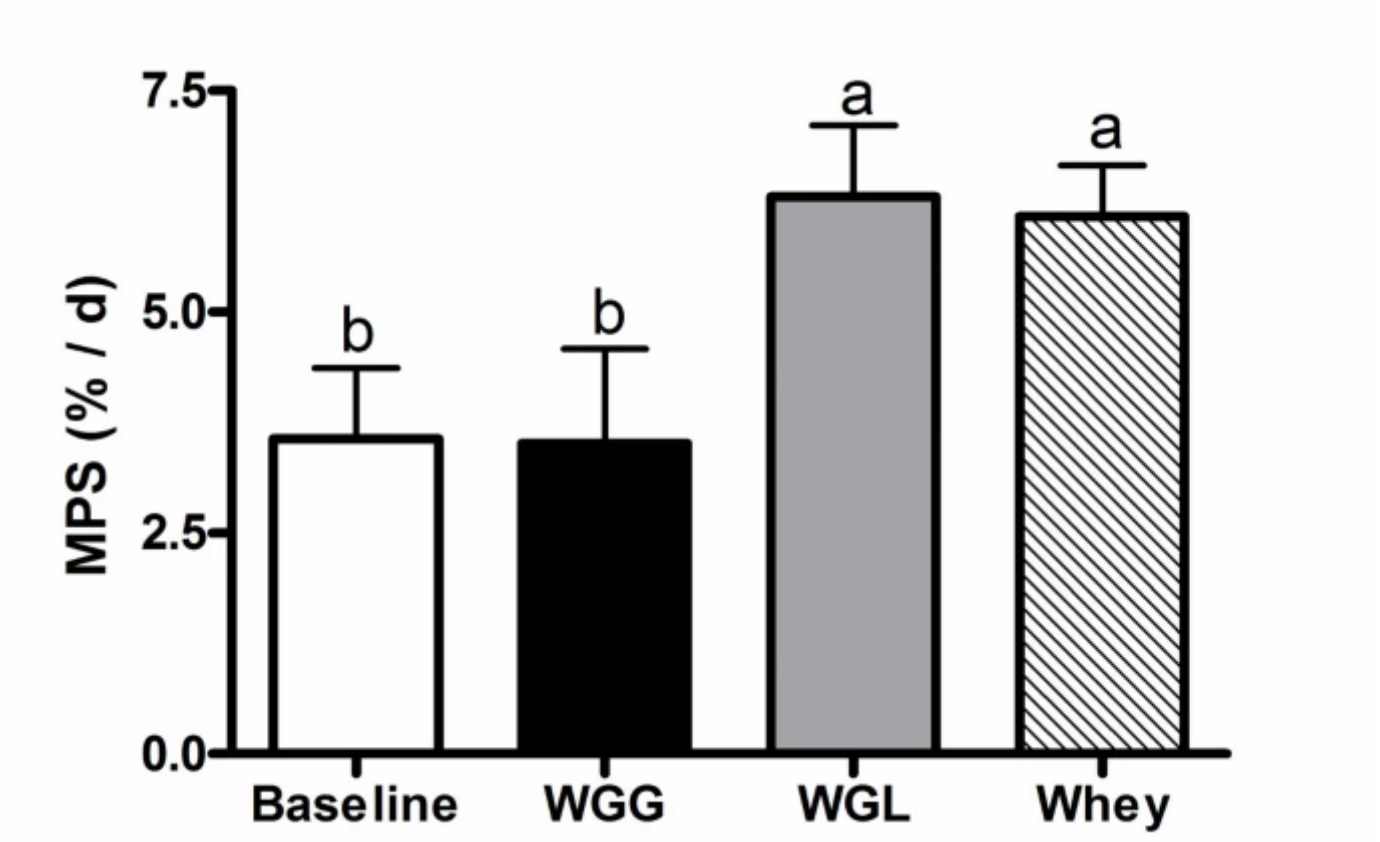
Experiment 2 compared meals containing wheat gluten, wheat + Leu, or whey proteins on the potential to increase plasma Leu and MPS over a 135-min time-course. At all time-points, wheat + Leu and whey groups had greater increases in plasma Leu

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than wheat or food-deprived controls; but wheat + Leu and whey were not different from each other (Table 5). Consistent with the plasma Leu changes, MPS increased in whey and wheat + Leu groups but not in the wheat group and were not different from each other (Figure 5).

Table 5 Experiment 2: Post-prandial changes for plasma amino acids [1-3]

Figure 5



Experiment 2 Muscle Protein Synthesis. Rates of protein synthesis in gastrocnemius muscle of rats consuming isonitrogenous meals containing wheat,wheat supplemented with leucine (Wheat + Leu),

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or whey protein. Data are means ± SEM; n = 5–6. Labeled means without a common letter differ, $P < 0.05$.

Plasma amino acid concentrations for the wheat and whey groups were consistent with Experiment 1. The wheat + Leu group was similar to wheat except Ile and Val concentrations tended to be lower in the Leu supplemented group. The time course patterns of change in plasma amino acids were similar for all groups. The amplitude of the post-prandial changes varied largely according to the amino acid content of the diet with the highest concentrations observed at 30 min declining at 90 and 135 min.

Post-prandial insulin concentrations at 30 min were greater in whey and wheat + Leu groups compared with the wheat group (Table 5). Akt signaling tended to increase from fasted at 30 min in both whey and wheat + leu groups but did not reach statistical significance at any time point (data not shown).

Discussion

Leu is recognized as a signaling factor for translation initiation and MPS [5, 18, 20]. However, the majority of this research has been done with large acute doses of free Leu, and the significance of this signal in physiological meals with different dietary proteins remains largely unknown. Accordingly, the present study examined responses of plasma amino acids, translation initiation factors, and MPS in adult rats fed complete meals with protein sources selected for

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differences in Leu content. This study demonstrated that in small meals with limited protein intake, MPS was stimulated in proportion to activation of translation factors and further establishes a threshold requirement for a minimum Leu content within a meal.

The relationship between post-prandial plasma Leu concentrations and stimulation of MPS has been previously observed with both animal [7, 21] and human [22, 23] studies. However, other studies reported using supplemental Leu producing substantial increases in plasma Leu that had no incremental effect on MPS [24–26]. While these studies appear contradictory, taken together, these studies are consistent with the hypothesis that Leu acts as a threshold trigger for activation of translation initiation. As plasma Leu increases after a meal from reduced fasted concentrations to post-prandial peak values, the change in concentration is recognized by the mTORC1 signal complex and triggers initiation. Once the threshold for activation has been achieved and the active ribosome complex assembled, additional increments in plasma Leu have minimal effects [5, 7].

Norton *et al.*[7] conducted a dose response study examining wheat and whey proteins fed at 10%, 20% and 30% of dietary energy. At isonitrogenous intakes, whey protein produced higher levels of plasma Leu, mTOR signaling, and MPS than wheat. However, MPS was not different comparing meals with 30% wheat vs. 20% whey consistent with the net Leu

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available from wheat (6.8% Leu) and whey (10.9% Leu). Similar support for Leu as a signal to initiate an anabolic meal response has been observed for young men consuming whey, soy and casein after exercise [23] and in older men or rats consuming whey versus casein [21, 22].

To further test the physiological significance of the threshold hypothesis, Experiment 1 utilized a meal-feeding protocol with adult rats. Based on previous research with rats of the same age and size [7] we estimated that the Leu required to achieve the initiation signal threshold at a meal would be ~50 to 60 mg of Leu. We selected 4 proteins that provided a range of Leu contents and designed a meal to provide 20% of daily energy and 16% of energy as protein. This combination of meal size and protein content allowed the 4 proteins to bracket the proposed Leu threshold (ie. wheat 46 mg, soy 54 mg, egg 60 mg, and whey 74 mg of Leu). The whey and egg proteins provided sufficient Leu to increase plasma Leu concentrations, increase phosphorylation of translation factors S6K1 and 4E-BP1, and stimulate MPS. These findings support a concept for a minimum meal threshold for Leu to stimulate post-prandial MPS.

While dietary Leu appears to be a critical factor to account for the post-meal MPS response, this does not exclude potential contributions from other amino acids. There is evidence that orally administered isoleucine (Ile) also activates mTORC1 signaling, although not as potent as Leu [18]. In complete

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proteins, Ile is typically present in proportion to the Leu content, allowing the possibility that Ile may in part contribute to the mTORC1 signaling and MPS. However, in the current study, rats fed soy significantly increased post-prandial plasma Ile concentration with no corresponding increase in mTORC1 signaling or MPS. This is consistent with previous research that only Leu among the EAA was able to increase MPS when infused in rats [27]. In the present study with complete meals, only the post-prandial changes in plasma Leu predicted changes in mTORC1 signaling and stimulation of MPS.

Beyond Leu and Ile contents, dietary proteins differ in other characteristics including distribution of other indispensable amino acids, differences in gastric emptying or digestibility, and insulinogenic properties. Experiment 2 was designed to isolate the importance of Leu within a meal and to test the significance of Leu content versus other intrinsic properties of protein sources. Experiment 2 demonstrated that supplementing the wheat protein meal with Leu to equalize the Leu content of the meals (wheat + Leu = whey) produced similar post-prandial patterns for plasma Leu and MPS response. Further, for each of the treatment groups, peak plasma Leu concentration occurred 30 min after the meal, and there was a strong correlation with Leu content of the meal and peak plasma Leu concentration ($r = 0.919$; $P < 0.05$). These findings are consistent with the post-exercise data of Churchward-Venne et al. [28] demonstrating that a suboptimal dose of whey protein (6.25 g)

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supplemented with Leu could produce the same MPS rate as a high dose of whey protein (25 g). These studies confirm that between different protein sources Leu content is an important predictor of post-meal MPS responses.

Also supporting the Leu threshold hypothesis, there are numerous examples of saturation of the Leu signal [5, 7, 19]. Using oral doses of free Leu ranging from 0.068 g/kg body weight up to 1.35 g/kg, Crozier et al. [5] demonstrated that the maximum rate of MPS was achieved in a 200 g rat at ~67 mg of Leu and that this represented maximum activation of translation initiation signals. Increasing dosage of Leu to double and triple this amount proportionally increased plasma Leu concentration but had no additional effects on initiation factors or MPS. Likewise, Debras et al. [24] observed no difference in MPS after a casein-based meal containing 81 mg of Leu versus casein plus Leu supplement with 237 mg of Leu; Norton et al. [7] found that a meal with 30% of energy from wheat gluten was similar to a 20% whey protein meal in producing maximum MPS and translation initiation responses; and Anthony et al. [19] reported that rats fed either a 20% soy or 20% whey protein meal after exercise both increased plasma Leu to approximately double baseline concentrations and both diets fully stimulated mTORC1 signaling and MPS. In total, these studies demonstrate that Leu is an essential threshold signal for translation initiation but additional amounts above the threshold do not produce additive effects on activation.

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The current studies demonstrate that insulin does not differentiate regulation of post-prandial MPS in adult rats consuming that same amounts of total carbohydrates and protein. Prior research suggests that insulin plays a permissive role in Leu-dependent stimulation of protein synthesis and translation initiation [8]. Consistent with this view, all protein groups in the current studies exhibited post-prandial increases in insulin and/or the down-stream signal element Akt-phosphorylation allowing for a permissive anabolic response to the meal, however the precise insulin concentration or the activation state of Akt had no apparent relationship to absolute rates of MPS. In the current studies, the post-prandial insulin response was relatively small presumably due to meal-feeding protocol and restricted carbohydrate load of the test meals. In Experiment 2, the insulin response was greatest 30 min post-meal in the whey and wheat + Leu groups relative to baseline control and wheat groups consistent with increases in MPS. While the test meals contained equal quantity and quality of carbohydrates and equal amounts of protein, the heightened insulin response in whey protein and wheat + Leu fed rats may contribute to greater rates of mTOR signaling and MPS. The specific contribution of insulin to MPS regulation in adults remains to be fully elucidated.

Conclusions

This research demonstrates that when protein is limited within the context of a small meal stimulation

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of MPS is dependent on the availability of sufficient Leu to initiate translation. Leu serves as a signal to facilitate assembly of the translation initiation complex [2]. Leu serves as a trigger to allow MPS to transition from a depressed state characterized with inhibition of translation factors 4E-BP1 and S6K1 after an overnight fast, to an active period of MPS. Hence, while the total daily protein intake may satisfy dietary guidelines, individual small meals with limited protein quantity and proteins with low Leu content may be inadequate to initiate the assembly process and stimulate MPS. This research supports the hypothesis that the Leu content of dietary proteins is important for muscle protein synthesis and highlights the need for long-term studies examining the impact of Leu density on muscle health and body composition.

Abbreviations

4E-BP1:
Eukaryotic initiation factor 4E binding protein-1

AMPK:
AMP Kinase

EAA:
Essential amino acids

eIF:
Eukaryotic initiation factor

FSR:
Factional rate of protein synthesis

Leu:

Leucine

Ile:

Isoleucine

MPS:

Skeletal muscle protein synthesis

mTOR:

Mammalian target of rapamycin

S6K:

Ribosomal protein p70 S6 Kinase.

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Competing interests

L.E. Norton scientific consultant to Scivation Inc., G.J. Wilson, C.J. Moulton, and P.J. Garlick, no conflicts of interest. D.K. Layman participates in NDC and National Cattleman's Beef Association speaker bureaus and serves on ENC Scientific Advisory Panel.

Authors' contributions

LEN, GJW, CJM, and DKL designed research; LEN, GJW, CJM, and PJG conducted research; LEN analyzed data; LEN, GJW, CJM, and DKL wrote the paper. LEN had primary responsibility for final content. All authors read and approved the final manuscript.

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Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Nutritional Physiological Phenomena
Tree Number(s)	G07.203.650
Unique ID	D009747
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D009747
Annotation	general or unspecified; prefer specifics; NUTRIENTS (see FOOD) and DIET are also available; use NUTRITIONAL SCIENCES for clinical nutrition as a specialty; use NUTRITION THERAPY or specifics for nutritional intervention
Scope Note	The processes and properties of living organisms by which they take in and balance the use of nutritive materials for energy, heat production, or building material for the growth, maintenance, or repair of tissues and the nutritive properties of FOOD.
Entry Term(s)	Nutrition Phenomena Nutrition Physiological Concepts Nutrition Physiological Phenomena Nutrition Physiological Phenomenon Nutrition Physiology Nutrition Process Nutrition Processes Nutritional Phenomena Nutritional Physiological Phenomenon Nutritional Physiology Nutritional Physiology Concepts Nutritional Physiology Phenomena Nutritional Physiology Phenomenon Nutritional Process Nutritional Processes
See Also	Diet Digestive System Physiological Phenomena

	Food
	Nutritional and Metabolic Diseases
Public MeSH Note	2009; see NUTRITION PHYSIOLOGY 2007-2008, see NUTRITION 1963-2006; for NUTRITION PHENOMENA see NUTRITION PHENOMENA 2007-2008; for NUTRITION PROCESSES see NUTRITION PROCESSES 2007-2016
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MeSH Heading	Diet
Tree Number(s)	G07.203.650.240
Unique ID	D004032
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D004032
Annotation	general or unspecified; prefer specific diets; DIET THERAPY and specifics are also available; /trends: do not confuse with DIET FADS; in veterinary journals consider also ANIMAL FEED; SILAGE; etc.
Scope Note	Regular course of eating and drinking adopted by a person or animal.
See Also	Cooking
Entry Combination	therapeutic use:Diet Therapy
Date Established	1966/01/01
Date of Entry	1999/01/01
Revision Date	2015/06/29

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Attachment 40e

Metabolism MeSH Descriptor Data 2022

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading	Metabolism
Tree Number(s)	G03
Unique ID	D008660
RDF Unique Identifier	http://id.nlm.nih.gov/mesh/D008660
Annotation	general only as metabolic process/phenomena irrespective of substance; prefer /metab with organs, organisms, diseases, substances
Scope Note	The chemical reactions in living organisms by which energy is provided for vital processes and activities and new material is assimilated.
Entry Version	METAB
Entry Term(s)	Anabolism Catabolism Metabolic Concepts Metabolic Phenomena Metabolic Phenomenon Metabolic Process Metabolic Processes Metabolism Concepts Metabolism Phenomena Process, Metabolic Processes, Metabolic
Public MeSH Note	TISSUE METABOLISM was heading 1963-65
History Note	METABOLIC PROCESSES was NON MESH 1992-97; TISSUE METABOLISM was heading 1963-65
Date Established	1960/01/01
Date of Entry	1999/01/01
Revision Date	2016/07/01

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Attachment 41a

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

ScienceDirect

Handbook of Pharmaceutical Wet Granulation
Theory and Practice in a Quality by Design Paradigm

2019, Pages 317-349

Chapter 9 - Binders in Wet Granulation

Thomas Dürig, Kapish Karan

Ashland LLC, Wilmington, DE, United States

Available online 5 October 2018, Version of Record 5 October 2018.

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https://doi.org/10.1016/B978-0-12-810460-6.00010-5 Get rights and content

Abstract

Binders or “granulating agents” are a critical formulation component in wet granulation, ensuring appropriate surface wetting ability to ensure adhesion and cohesion between inter-particulate surfaces in the wet state, as well as excellent plasticity, compactibility and binding ability. Traditionally binder selection has been empirical however advances have been made in mechanistic and quantitative particle engineering and materials science. The purpose of this chapter is to review the physical-chemical properties of the major binders in current pharmaceutical and commercial use, and to provide a practical and logical guide to wet binder selection and use in the context of physical-chemical properties of the substrate and the binder. Recent advances in mechanistic understanding of granulation technology will be discussed in detail, specifically the importance of selecting binders with a focus on surface energetics, wetting, spreadability, and mechanical properties of the system.

Previous Next

Keywords

Binders; Wet granulation; Polymers; Viscosity; Wettability; Surface energetics; Stability; Solvent; Regulatory

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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2022, Applied Food Research

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Handbook of pharmaceutical wet granulation : theory and practice in a quality by design paradigm

Authors: Ajit S Narang (Editor), Sherif I F Badawy (Editor)

Print Book 2018

Amsterdam : Academic Press, 2018.

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U.S. Patent No. 11,224,241

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QV 779 N218h 2018

More Author/Title Info: edited by Ajit S. Narang, Sherif I.F. Badawy.

Physical Description: 1 volume ; 28 cm

Subjects:

Faceted Application of Subject Terminology

Handbooks and manuals

Library of Congress Subject Headings

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Granulation

Medical Subject Headings

Technology, **Pharmaceutical**

Drug Compounding

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Particle Size

Wetting Agents

Répertoire de Vedettes-Matière

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Attachment 41c

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245 00 Handbook of pharmaceutical wet granulation : $b theory and practice in a quality by design paradigm / $c edited by Ajit S. Narang, Sherif I.F. Badawy.
264 1 Amsterdam : $b Academic Press, $c 2018.
300 1 volume ; $c 28 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
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008 860211i| anannbabn |b ana
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HEADING: Granulation

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040 __ |**a** DLC |**c** DLC
150 __ |**a** Granulation
550 __ |**w** g |**a** Agglomeration
550 __ |**w** g |**a** Manufacturing processes
670 __ |**a** Work cat.: Granulation technology for bioproducts, 1990.
670 __ |**a** Hackh chem. dict.: |**b** 5-e.
670 __ |**a** TEST |**b** (Granulation UF Graining)
670 __ |**a** Random House.
670 __ |**a** Web. 3.
906 __ |**t** 9026 |**u** fk05 |**v** 1
952 __ |**a** 1 bib. record(s) to be changed
952 __ |**a** LC pattern: Agglomeration; Pelletizing
953 __ |**a** ff01

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Handbook of Pharmaceutical Wet Granulation Edition 1 .

Type of Work: Text
Registration Number / Date: TX0008830249 / 2019-12-06
Application Title: Handbook of Pharmaceutical Wet Granulation Edition 1 .
Title: Handbook of Pharmaceutical Wet Granulation Edition 1 .
Description: Book, 859 p.
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Date of Creation: 2018
Date of Publication: 2018-10-09
Nation of First Publication: United States
Authorship on Application: Sherif Badawy; Citizenship: not known. Authorship: text.

Ajit Narang; Citizenship: not known. Authorship: text.

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ISBN: 9780128104606

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